STABILITY PROFILING OF ORMELOXIFENE HYDROCHLORIDE USING ACCELERATED TESTING AS PER ICH GUIDELINES

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Abstract

Pharmaceutical stability testing plays a crucial role in ensuring the safety, efficacy, and quality of drug products throughout their shelf life. Analytical techniques are widely used in pharmaceutical industries for the qualitative and quantitative estimation of active pharmaceutical ingredients and degradation products. The present study was aimed at the development and validation of a simple, precise, and stability-indicating Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method for the estimation of Ormeloxifene Hydrochloride in tablet dosage form. Chromatographic separation was achieved using a C18 column with a mobile phase consisting of Acetonitrile and Tetra methyl ammonium hydroxide (80:20 v/v) adjusted to pH 7.6. Detection was carried out at 280 nm using a UV detector. Accelerated stability studies were performed according to ICH guidelines at storage conditions of 40°C ± 2°C and

75% ± 5% RH for a period of six months. The assay results indicated that the drug remained stable within acceptable limits throughout the study period. The developed method was found to be accurate, precise, reproducible, and suitable for routine quality control analysis and stability studies of Ormeloxifene Hydrochloride tablets.

Keywords: Ormeloxifene Hydrochloride, RP-HPLC, Stability studies, Accelerated stability testing, Pharmaceutical analysis.

INTRODUCTION

Stability testing is termed as a complex process because of involvement of a variety of factors influencing the stability of a pharmaceutical product. Stability testing of pharmaceutical products is a complex set of procedures involving considerable cost, time consumption and scientific expertise in order to build in quality, efficacy and safety in a drug formulation. Scientific and commercial success of a pharmaceutical product can only be ensured with the understanding of the drug development process and the myriad tasks and milestones that are vital to a comprehensive development plan. The most important steps during the developmental stages include pharmaceutical analysis and stability studies that are required to determine and assure the identity, potency and purity of ingredients, as well as those of the formulated products **(Singh et al., 2000).**

The chemical reactions like hydrolysis, oxidation, reduction, racemization etc. that occur in the pharmaceutical products may lead to the formation of degradation product, loss of potency of active pharmaceutical ingredient (API), loss of excipient activity like antimicrobial preservative action and antioxidants etc. **(Carstensen et al., 2000).**

Stability of a pharmaceutical product can also be affected because of microbiological changes like growth of microorganisms in non-sterile products and changes in preservative efficacy **(Matthews *et al.*, 1999).**

Important of stability testing

1. Ensures Drug Safety and Efficacy

- Stability testing helps determine how long a drug maintains its intended effectiveness and remains safe for use.
- It prevents the distribution of degraded or harmful products to consumers.

2. Determines Shelf Life and Expiry Date

- The data from stability testing help manufacturers establish the product's shelf life and storage conditions.
- It ensures that drugs retain their potency until the expiration date.

3. Compliance with Regulatory Requirements

- Regulatory agencies like the FDA (U.S.), EMA (Europe), and ICH (International Council for Harmonisation) require stability data before approving a drug for market distribution.

  Non-compliance can lead to product recalls, fines, or rejections.

4. Identifies Potential Degradation Products

- Over time, drugs may degrade into harmful by-products. Stability testing identifies these degradants and ensures they remain within acceptable limits.

5. Guides Storage and Packaging Decisions

- It helps determine the appropriate storage conditions (e.g., refrigeration, room temperature).Assists in selecting packaging materials that protect the drug from environmental factors.

6. Supports Product Development and Formulation

- Stability studies guide pharmaceutical scientists in optimizing formulations to enhance drug stability.
- Ensures product consistency across different batches.

Regulatory Guidelines for Stability Testing

A. ICH Guidelines: ICH Q1A(R2) provides general stability requirements, while other guidelines (Q1B-Q1E) address specific aspects like photo stability and bracketing designs.

Table 1: ICH Guidelines For Stability Studies

ICH Codes	Guidelines title
Q1A	Stability testing of new drug substances and products (second revision)
Q1B	Photo stability testing of new drug substances and products
Q1C	Stability testing of new dosage form
Q1D	Bracketing and Matrixing Designs for the stability testing of drug substances and products
Q1E	Evaluation of stability data
Q1F	Stability data package for registration applications in climatic zones III and IV

B. US FDA: Aligns with ICH standards but includes additional guidance for accelerated stability studies.

C. WHO: Focuses on harmonized global stability requirements, particularly for medicines intended for developing regions.

The WHO stability testing guidelines are aligned with ICH but also provide additional recommendations for developing countries. They classify climate zones into four categories, similar to ICH guidelines, and emphasize testing for different regions. WHO requires bracketing and matrixing approaches to optimize testing while reducing the number of samples needed for large batches.

European Medicines Agency (EMA): Emphasizes climatic zone-based stability protocols.

High Performance Liquid Chromatography

HPLC is an advanced chromatographic technique used for separation, identification, and quantification of components present in a mixture. It operates based on adsorption or partition principles depending on the stationary phase used.

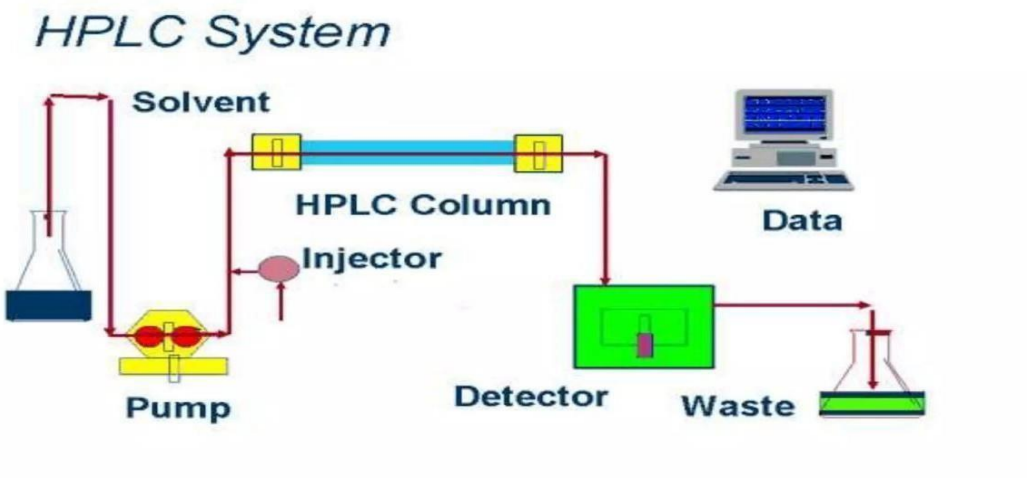


Figure 1: Schematic representation of High performance liquid chromatography

Advantages

- High sensitivity
- High resolution
- Rapid analysis
- Suitable for non-volatile compounds

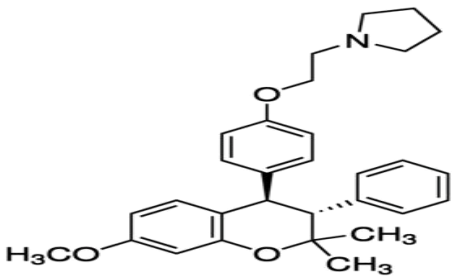
Instrumentation

- Pump
- Injector
- Column
- Detector
- Data acquisition system

Applications

- Assay of drugs
- Impurity profiling
- Stability studies

DRUG PROFILE OF ORMELOXIFENE HYDROCHLORIDE



Ormeloxifene Hydrochloride is a non-steroidal selective estrogen receptor modulator used as an oral contraceptive and for treatment of dysfunctional uterine bleeding.

- Molecular formula: $C_{30}H_{35}NO_3 \cdot HCl$
- Molecular weight: 494.07 g/mol
- Appearance: White crystalline powder
- Half-life: 165–170 hours
- Mechanism: Anti-estrogenic action on endometrium preventing implantation

MATERIALS AND METHODS

Instruments

- Agilent HPLC 1100 series with UV detector
- Analytical balance
- Ultrasonicator
- Vacuum filtration unit

Chemicals

- Acetonitrile (HPLC grade)

- Tetra methyl ammonium hydroxide
- Ortho phosphoric acid
- Purified water

Chromatographic Conditions

- Column: C18 (250 × 4.6 mm, 5 µm)
- Mobile phase: Acetonitrile : TMAH (80:20 v/v, pH 7.6)
- Flow rate: 1.5 mL/min
- Detection wavelength: 280 nm
- Injection volume: 20 µL
- Run time: 10 minutes

Preparation of Standard Solution

Accurately weighed 30 mg of Ormeloxifene Hydrochloride standard was transferred into a 100 mL volumetric flask and dissolved in mobile phase to obtain a concentration of 300 µg/mL

Preparation of Sample Solution

Twenty tablets were weighed and powdered. An amount equivalent to 30 mg drug was transferred into a volumetric flask and diluted with mobile phase to obtain 300 µg/mL solution.

RESULTS AND DISCUSSIONS

A stability-indicating reverse-phase high-performance liquid chromatographic (RP-HPLC) method was developed for the quantitative estimation of ormeloxifene hydrochloride in tablet dosage form. The optimized chromatographic conditions provided good resolution with a retention time of about 8.6 minutes and a total run time of 15 minutes. Detection at 280 nm ensured maximum drug absorbance with minimal interference from excipients and degradation products. System suitability testing performed as per USP <621> and ICH Q2(R1) guidelines showed peak area variability

within ±2%, confirming adequate system performance.

The method demonstrated good accuracy and precision, as the assay values were in close agreement with the labeled claim. Validation studies conducted according to ICH guidelines confirmed the reliability of the analytical procedure. Accelerated stability studies carried out at initial, 1-month, 3-month, and 6-month intervals showed percentage assay values ranging from 97.42% to 94.30%, indicating no significant degradation of the drug. Low %RSD values further established method reproducibility. Overall, the developed RP-HPLC method was found to be specific, robust, and suitable for routine quality control and stability assessment of ormeloxifene hydrochloride formulations.

Initial Assay for Stability Studies

Table 2: Assay for ormeloxifene HCl results (Initial month)

BRAND	**% AMOUNT FOUND**
Ormeloxifene HCl	97.24%

System parameters

System was performed by injecting three replicate injections of drug into the system to observe sharp peaks of Ormeloxifene HCl at retention times 2.40 min, respectively in reference to the standard solution and to determine the test concentration.

ORMELOXIFENE HCl STANDARD

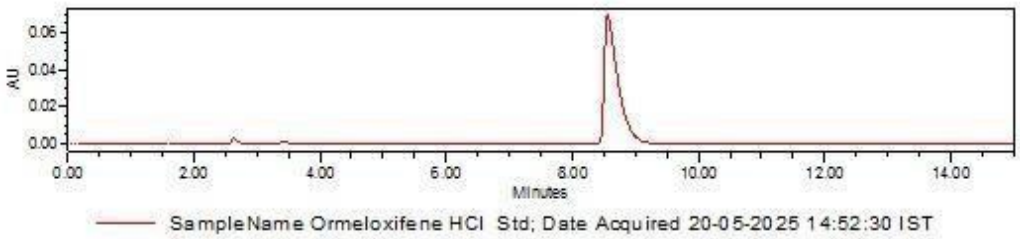


Figure 2: Chromatogram for Ormeloxifene HCl Standard 1 (Initial month)

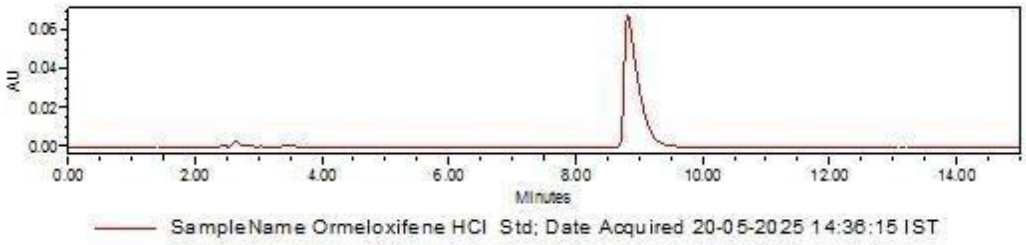


Figure 3: Chromatogram for Ormeloxifene HCl Standard 2 (Initial month)

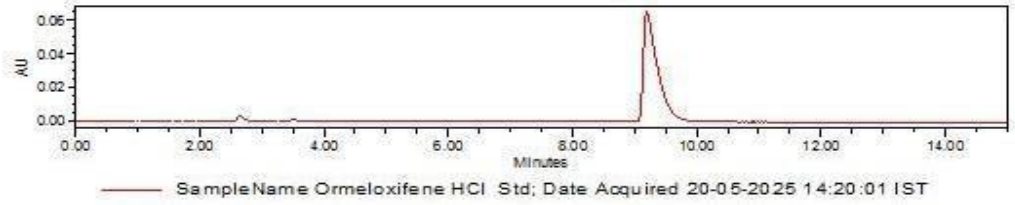


Figure 4: Chromatogram for Ormeloxifene HCl Standard 3 (Initial month)

Table 3: The result of chromatographic study for the Ormeloxifene HCl standard (Initial month)

S.NO	Standard Area Ormeloxifene HCl	RT
1	1051902	8.561
2	1045689	8.821
3	1034698	8.596
Mean	1044096.0	8.6

ORMELOXIFENE HCl SAMPLE

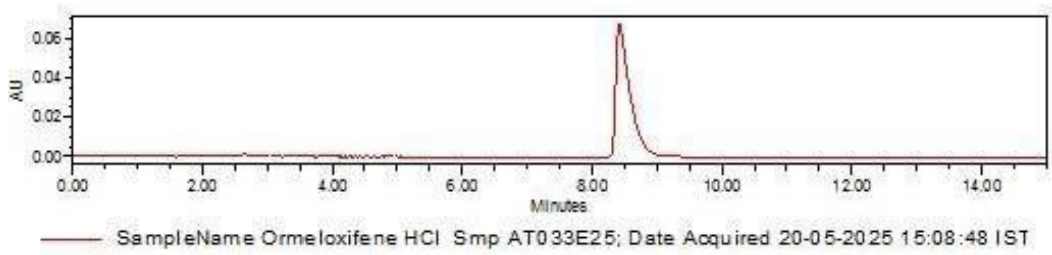


Figure 5: Chromatogram for Ormeloxifene HCl Sample 1 (Initial month)

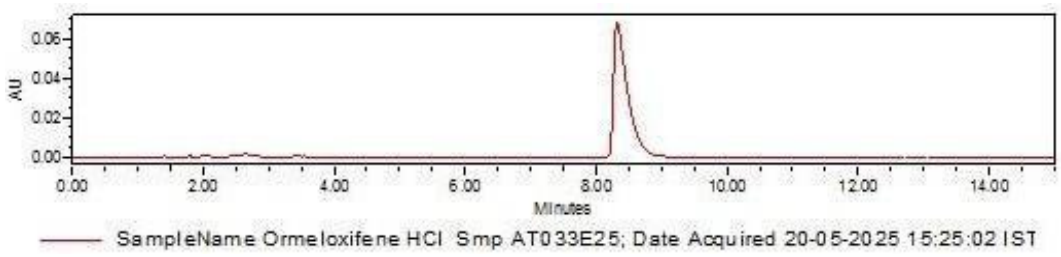


Figure 6: Chromatogram for Ormeloxifene HCl Sample 2 (Initial month)

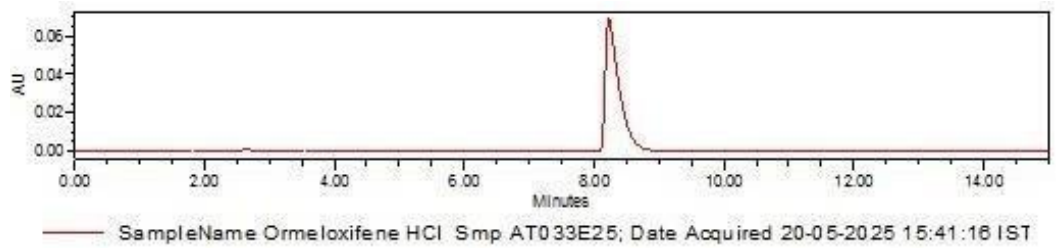


Figure 7: Chromatogram for Ormeloxifene HCl Sample 3 (Initial month)

Table 4: The result of chromatographic study for the Ormeloxifene HCl sample (Initial month)

S.NO	Sample Area Ormeloxifene HCl	RT
1	10119033	8.230
2	1017102	8.322
3	1009763	8.421
Mean	1015299.4	8.3

First Month Assay for Stability Studies

Table 5: Assay for ormeloxifene HCl results (First Month)

BRAND	% AMOUNT FOUND
Ormeloxifene HCl	94.58%

System parameters

System was performed by injecting three replicate injections of drug into the system to observe sharp peaks of Ormeloxifene HCl at retention times 2.44 min, respectively in reference to the standard solution and to determine the test concentration.

ORMELOXIFENE HCl STANDARD

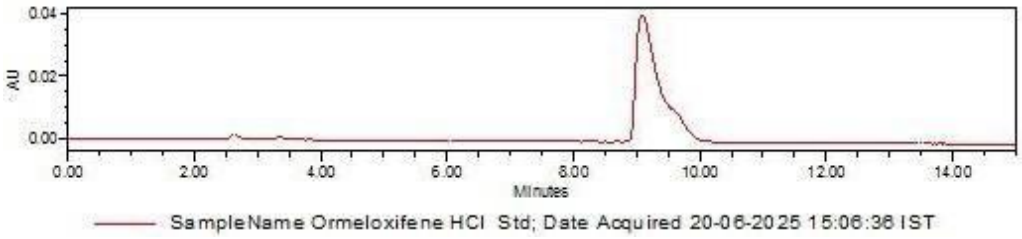


Figure 8: Chromatogram for Ormeloxifene HCl Standard 1 (First Month)

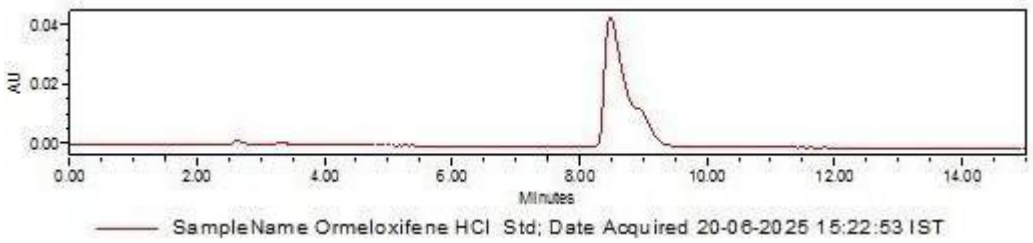


Figure 9: Chromatogram for Ormeloxifene HCl Standard 2 (First Month)

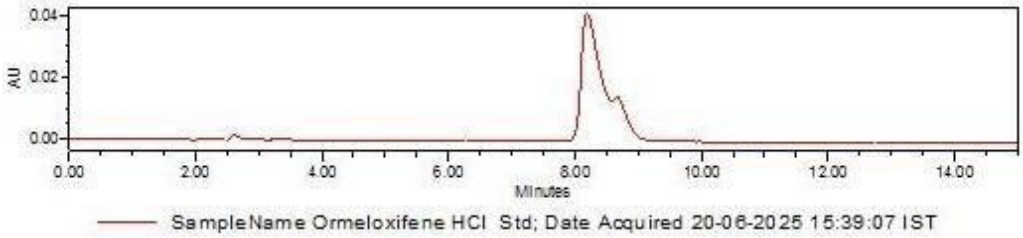


Figure 10: Chromatogram for Ormeloxifene HCl Standard 3 (First Month)

Table 6: The result of chromatographic study for the Ormeloxifene HCl standard (First Month)

S.NO	Standard Ormeloxifene HCl	Area	RT

1	1061656	8.192
2	1068854	8.486
3	1062334	9.091
Mean	1064281.0	8.6

ORMELOXIFENE HCl SAMPLE

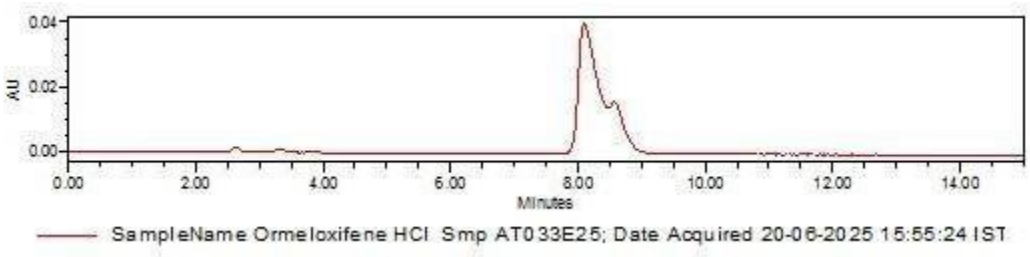


Figure 11: Chromatogram for Ormeloxifene HCl Sample 1 (First Month)

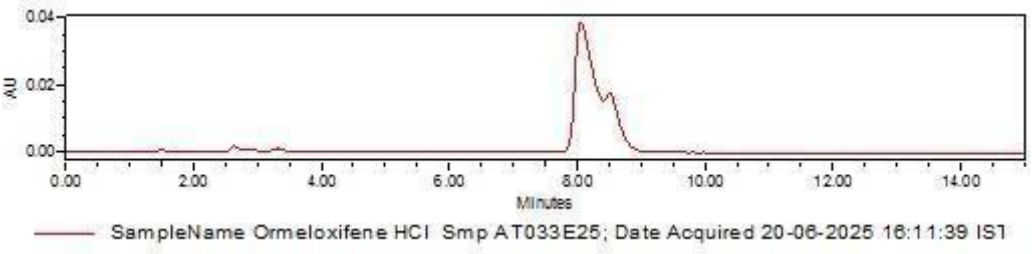


Figure 12: Chromatogram for Ormeloxifene HCl Sample 2 (First Month)

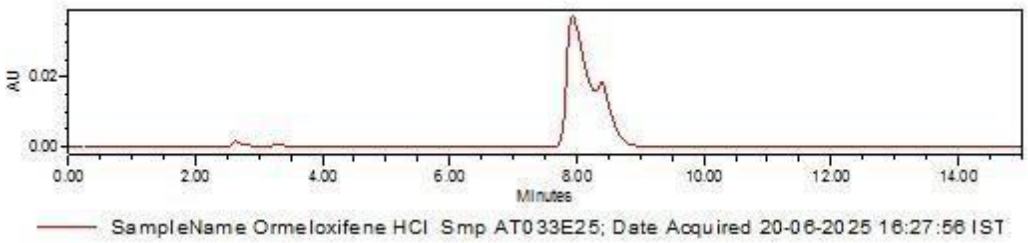


Figure 13: Chromatogram for Ormeloxifene HCl Sample 3 (First Month)

Table 7: The result of chromatographic study for the Ormeloxifene HCl sample (First Month)

S.NO	Sample Area Ormeloxifene HCl	RT
1	1001447	7.930
2	1016390	8.060
3	1002132	8.101
Mean	1006656.5	8.0

Third Month Assay for Stability Studies

Table 8: Assay for ormeloxifene HCl results (Third Month)

BRAND	% AMOUNT FOUND
Ormeloxifene HCl	93.68%

System parameters

System was performed by injecting three replicate injections of drug into the system to observe sharp peaks of Ormeloxifene HCl at retention times 2.40 min, respectively in reference to the standard solution and to determine the test concentration.

ORMELOXIFENE HCl STANDARD

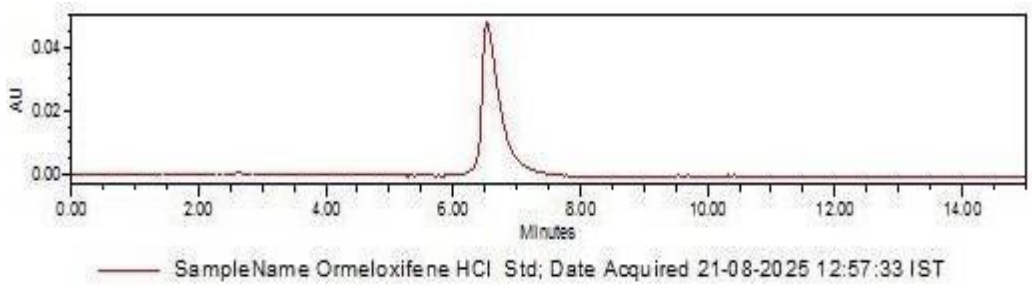


Figure 14: Chromatogram for Ormeloxifene HCl Standard 1 (Third Month)

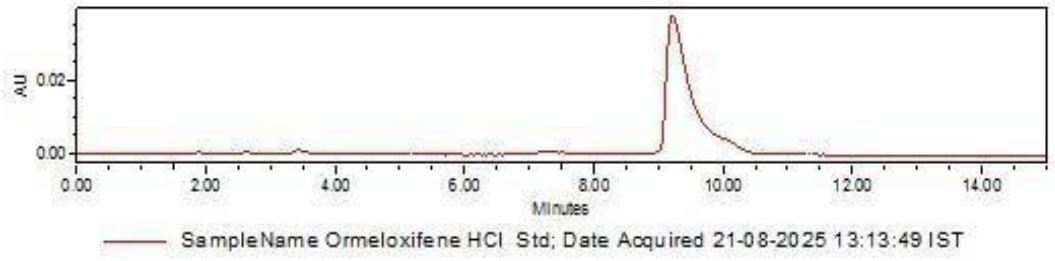


Figure 15: Chromatogram for Ormeloxifene HCl Standard 2 (Third Month)

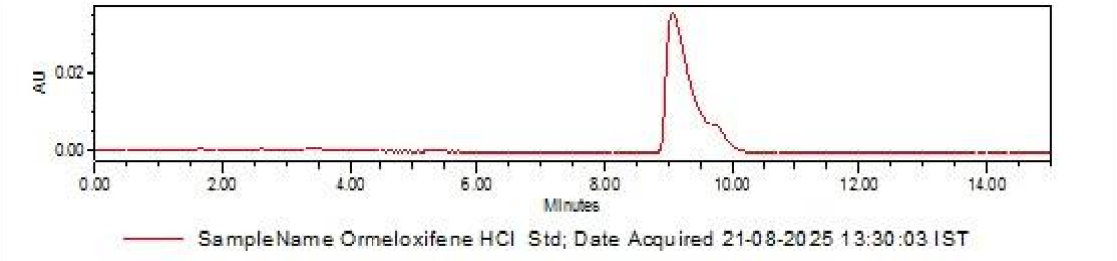


Figure 16: Chromatogram for Ormeloxifene HCl Standard 3 (Third Month)

Table 9: The result of chromatographic study for the Ormeloxifene HCl standard (Third month)

S.NO	Standard Area Ormeloxifene HCl	RT
1	1023348	8.531
2	1030754	9.070
3	1022788	9.217
Mean	1025630.1	8.3

ORMELOXIFENE HCl SAMPLE

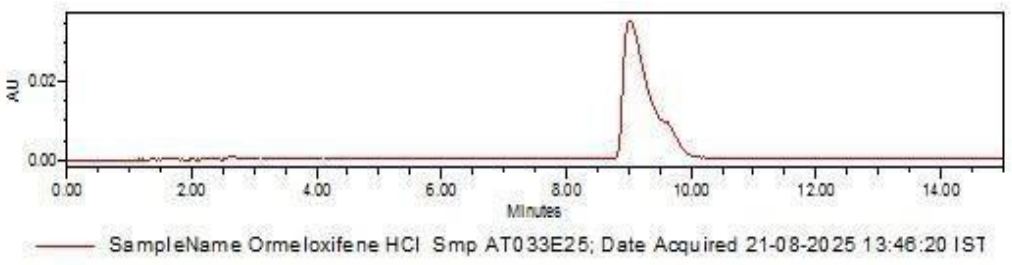


Figure 17: Chromatogram for Ormeloxifene HCl Sample 1 (Third Month)

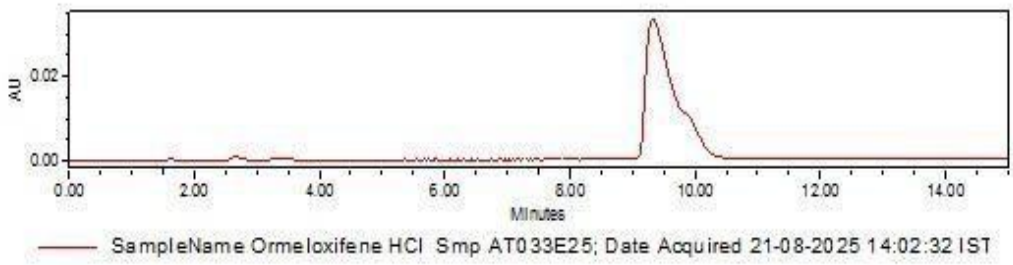


Figure 18: Chromatogram for Ormeloxifene HCl Sample 2 (Third Month)

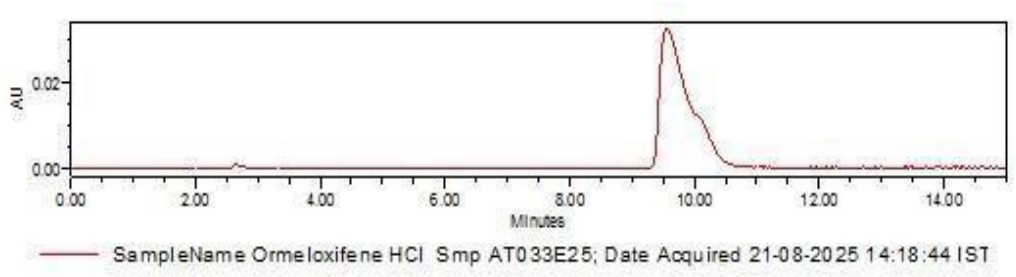


Figure 19: Chromatogram for Ormeloxifene HCl Sample 3 (Third Month)

Table 10: The result of chromatographic study for the Ormeloxifene HCl sample (Third Month)

S.NO	Sample Area Ormeloxifene HCl	RT
1	960452	9.020
2	960839	9.327
3	961078	9.558
Mean	960789.4	9.3

Sixth Month Assay for Stability Studies

Table 11: Assay for ormeloxifene HCl results (Sixth Month)

BRAND	% AMOUNT FOUND
Ormeloxifene HCl	94.30%

System parameters

System was performed by injecting three replicate injections of drug into the system to observe sharp peaks of Ormeloxifene HCl at retention times 2.40 min, respectively in reference to the standard solution and to determine the test concentration.

ORMELOXIFENE HCl STANDARD

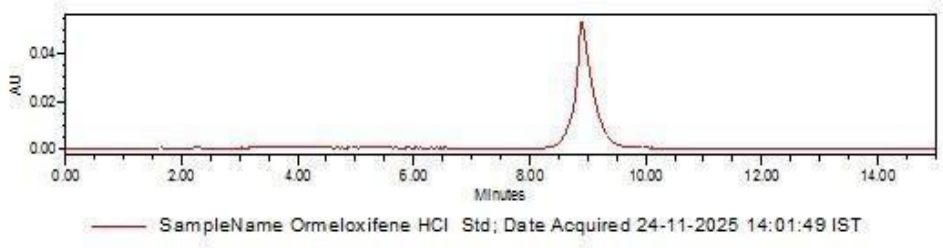


Figure 20: Chromatogram for Ormeloxifene HCl Standard 1 (Sixth Month)

Table 12: The result of chromatographic study for the Ormeloxifene HCl standard (Sixth Month)

S.NO	Standard Area Ormeloxifene HCl	RT
1	1100822	8.779
2	1101542	8.896
Mean	1101182.1	8.8

ORMELOXIFENE HCl SAMPLE

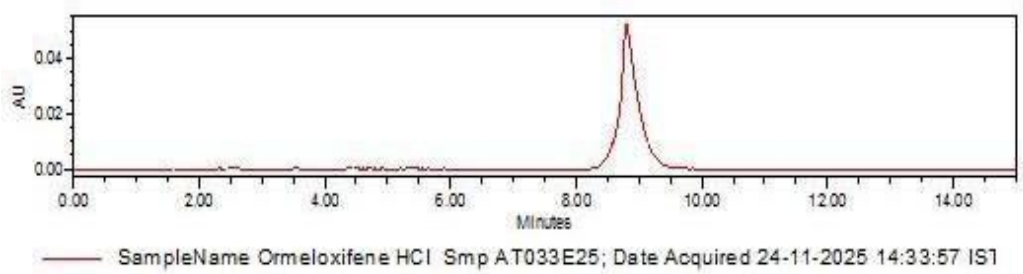


Figure 21: Chromatogram for Ormeloxifene HCl Sample 1 (Sixth Month)

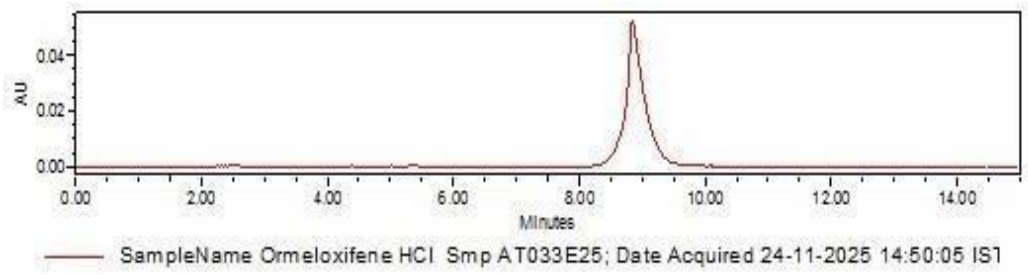


Figure 22: Chromatogram for Ormeloxifene HCl Sample 2 (Sixth Month)

Table 13: The result of chromatographic study for the Ormeloxifene HCl sample (Sixth Month)

S.NO	Sample Area Ormeloxifene HCl	RT
1	1038933	8.799
2	1038116	8.847
Mean	1038524.3	8.8

SUMMARY AND CONCLUSIONS

The present study evaluated the accelerated stability of Ormeloxifene hydrochloride tablets using a validated RP-HPLC method. A simple, precise, and reliable chromatographic method was developed with detection at 280 nm, showing a retention time of about 8–9 minutes and good resolution. Accelerated stability studies were conducted as per ICH guidelines at 40°C ± 2°C and 75% ± 5% RH, with analysis performed at initial, 1-month, 3-month, and 6-month intervals. The assay results showed only minor variations in drug content, and chromatograms exhibited well-defined peaks without significant interference from excipients or degradation products. The percentage assay values remained within acceptable pharmaceutical limits, confirming the chemical stability of the formulation under accelerated conditions.

Overall, the developed RP-HPLC method was found to be accurate, precise, reproducible, and suitable for routine quality control analysis and stability assessment

of Ormeloxifene hydrochloride tablet formulations. The study indicates that the product possesses adequate stability and acceptable shelf-life characteristics when stored under recommended conditions.

Table 14: Summary of Accelerated Stability Study for Ormeloxifene HCl

Specification	Initial	1st Month	3rd Month	6th Month
Description White colour round shape biconvex of uncoated tablets	Complies	Complies	Complies	Complies
Identification Should comply with standard	Complies	Complies	Complies	Complies
Average weight 200mg ± 7.5%	200.1 mg	200.3 mg	200.5 mg	200.6 mg
Disintegration time NMT: 15 Minutes	2' 40"	2' 44"	2' 48"	2' 52"
Assay: Each uncoated contains : Ormeloxifene HCl 30 mg – 27 mg to 33 mg (90% to 110%)	97.24%	94.58%	93.68%	94.30%
Analysis Date 1.Started date:20.05.2025 2.Completed date:24.11.2025	20.05.2025	20.06.2025	21.08.2025	24.11.2025

REFERENCE

1. **Ali J, et.al, 2008.** Dosage form and design.3rd ed. Delhi. Birla Publications Pvt. Ltd (2008) 100-123
2. **Anderson G, et.al, 1991.** Determination of product shelf life and activation energy for five drugs of abuse. Clin. Chem. 1991;37:398-402.
3. **August, et.al, 1988.** 18, 731–742.
4. **Arunachalam A, et.al, 2013.** Stability Studies. Asian Journal of Pharmaceutical Analysis and medicinal chemistry, 1(4): 184-195, (2013).

5. **Bhutani H, Mariappan TT.** Behaviour of Uptake of Moisture by Drugs and Excipients under Accelerated Conditions of Temperature and Humidity in the Absence and the Presence of light.
6. **Bott RF, Oliveira WP, 2007**. Storage conditions for stability testing of pharmaceuticals in hot and humid regions. Drug Dev. and Indus. Pharm. 2007;33:393401.
7. **Blake, J, et.al, 2017.** Evaluation of a Single-administration Ototopical Treatment for Canine Otitis Externa: A Randomised Trial. Vet. Rec. Open 2017, 4, e000219.
8. **Banik. S, et.al, 2015.** Bangladesh Pharm. J., 18, 163 (2015).
9. **Bansal G, et.al 2016.** Stability testing of herbal drugs: challenges, regulatory compliance and perspectives. Phytother Res. 2016;30:1046-1058. doi:10.1002/.
10. **Barrett J, et.al, 1973.** An examination of acid-base equilibria of 1,4 benzodiazepines by spectrophotometry. J Pharm Pharmacol25:387-393.
11. **Baired, R.M, et.al 2010.** Hand book of microbial quality control. p.22-36. www.ICH.org - assessed on 21 November 2010.
12. **C.K. Sekhar, et.al, 2013.** Int. J. Univ. Pharm. Bio. Sci., 2, 1 (2013).
13. **Carstensen JT, Rhodes CT, 2000.** Drug Stability, Principles and Practices, Marcel Dekker, New York (2000). Clin Res Drug Reg Affairs.1993;10:177–185.
14. **Cannors, et.al, 1979.** Chemical Stability of Pharmaceuticals‟. A handbook of Pharmacists. John Wiley & Sons, New York.
15. **Carstensen JT, Rhodes CT, et.al, 1993.** Drug Reg. Affairs. 1993;10:177-185
16. **Carstensen JT., 2000.** Drug Stability, Principles and Practices, Marcel Dekker, New York (2000)
17. **Cha J, et.al, 2001.** Stability studies in Handbook of modern pharmaceutical analysis. Separation Science and Technology. Elsevier (2001) 459-505
18. **Connors KA, et.al, 1973.** Chemical stability of pharmaceuticals-a handbook for pharmacists. New York: John Wiley & Sons (1973) 8-119.