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Original Article

Method Development and Forced Degradation Profiling of Abametapir by UV Spectrophotometry: A Stability-Indicating Investigation as per ICH

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A B S T R A C T

A zero-order derivative UV spectrophotometric method was developed to evaluate the forced degradation behavior of Abametapir at a concentration of 6 µg/mL, measured at λmax 311 nm. Stress conditions applied included acidic (0.1 N HCl), alkaline (0.1 N NaOH), oxidative (3% H_2O_2), thermal (80 °C dry heat), and photolytic (sunlight exposure for 12 hours). The method demonstrated high sensitivity, with a calculated limit of detection (LOD) and limit of quantification (LOQ) of 0.10962 µg/mL and 0.33218 µg/mL, respectively. Calibration curve data indicated excellent linearity in the range of 2–10 µg/mL, with a regression equation of Y = 0.0862x + 0.0875 and a correlation coefficient (R^2) of 0.9992. Recovery rates ranged from 98.17% to 101.6% across stress conditions, with oxidative degradation (50.67%) and alkaline hydrolysis (29.87%) causing the greatest reductions in absorbance. Minimal degradation was observed under thermal (6.21%), photolytic (7.88%), and acidic (11.56%) conditions. The method exhibits reproducibility, accuracy, and robustness, confirming its applicability for degradation studies and routine quality control of Abametapir.

Keywords: Abametapir, UV spectrophotometry, zero-order derivative method, forced degradation, ICH guidelines, stability studies.

1. Introduction

Abametapir, chemically known as *5,5'-dimethyl-2,2'-bipyridine*, is a topical pediculicidal agent approved by the U.S. Food and Drug Administration (FDA) in 2020 under the brand name Xeglyze[1]. Its mechanism of action involves the inhibition of metalloproteinases critical to louse embryogenesis and development, offering effective single-application treatment for head lice infestations in both adult and pediatric populations[2].

While high-end analytical techniques such as LC-ESI-MS/MS have been reported for the quantification of Abametapir in biological matrices[3], there remains a significant gap in simple, economical spectrophotometric methods for its analysis in bulk form. This presents challenges for academic and quality control laboratories where access to advanced chromatographic systems may be limited.

Ultraviolet-Visible (UV-Vis) spectrophotometry is a widely adopted tool in pharmaceutical analysis due to its simplicity, minimal sample preparation, affordability, and ease of validation[4]. Among its techniques, zero-order derivative spectrophotometry enhances sensitivity and specificity by resolving overlapping bands[5].

The present study aims to develop and validate UV spectrophotometric methods focused on zero-order calibration and forced degradation profiling of Abametapir.

Degradation behavior was evaluated under acidic, alkaline, oxidative, thermal, and photolytic stress conditions, in accordance with ICH guidelines, using

a fixed concentration of 6 µg/mL. Absorbance was recorded at 311 nm, with degradation analyzed through spectral shifts and recovery calculations.

This study proposes a simple, validated UV spectrophotometric method—based on zero-order derivative analysis at 311 nm—for assessing the stability of Abametapir through systematic forced degradation profiling under ICH-specified stress conditions[6].

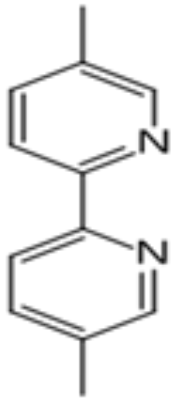

Figure 1. Chemical structure of Abametapir

2. Materials and Methods

2.1 Chemicals and Reagents

Abametapir (purity: 98.0%) was procured from Yarrow Chem Products Pvt. Ltd., Mumbai, India. The following analytical-grade reagents were employed in the forced degradation studies of Abametapir: Sodium hydroxide (NaOH) LR-grade flakes were used to simulate alkaline hydrolysis by preparing a 0.1 N solution according to standard protocol. Hydrochloric acid (HCl), 35–38% LR-grade, was appropriately diluted to 0.1 N to induce acidic degradation conditions. Oxidative stress was applied using hydrogen peroxide (H_2O_2), a 3% w/v LR-grade solution, obtained from Swathi & Co. serving as the oxidizing agent. Distilled water was used for reagent preparations and sample processing.

2.2 Instrumentation

A double-beam UV-VIS spectrophotometer (UV 1800, Shimadzu) was utilized, interfaced with a computer equipped with the UV probe 4.21 spectra management software, employing 10 mm quartz cells. The spectra were acquired using the following instrumental parameters: Wavelength range: 200 to 400 nm.

All weights were measured using an electronic balance (Model Shimadzu AUX 120). Spectral measurements were conducted with a UV-visible double-beam spectrophotometer fitted with matching 1 cm quartz cells. Absorbance was measured at 311 nm utilizing zero-order derivative mode. All data processing and curve fitting were conducted utilizing conventional spectrophotometric software UV probe 4.21.

2.3 Preparation of Standard and Working Solutions

Stock A was prepared by dissolving 100 mg of Abametapir in 100 mL of 0.1 N HCl, obtaining a concentration of 1000 µg/mL. To make Stock B, dilute 10 mL of Stock A with 100 mL of 0.1 N HCl (100 µg/mL). To make Stock C, dilute 20 mL of Stock B with 100 mL of 0.1 N HCl (20 µg/mL).

2.4 Selection of Analytical Wavelengths

Aliquots of 1–5 mL of Stock C (20 µg/mL) were individually diluted to 10 mL with 0.1 N HCl to obtain standard solutions of 2–10 µg/mL. The UV absorption spectra of Abametapir were measured between 200 and 400 nm using a UV-visible spectrophotometer (Shimadzu UV-1800 double-beam).

2.5 Method Development

A UV spectrophotometric method was developed for the estimation of Abametapir in bulk using the zero-order approach. The absorbance of the drug was measured directly at 311 nm. Method parameters such as wavelength selection, spectral resolution, and sensitivity were optimized. Calibration curves were constructed for concentrations ranging from 2 to 10 µg/mL.

2.6 Method Validation

The zero-order spectrophotometric method was validated in accordance with ICH Q2(R1) guidelines[7]. All validation parameters were performed using standard solutions of abametapir in bulk form, without the inclusion of any formulation or excipient matrix.

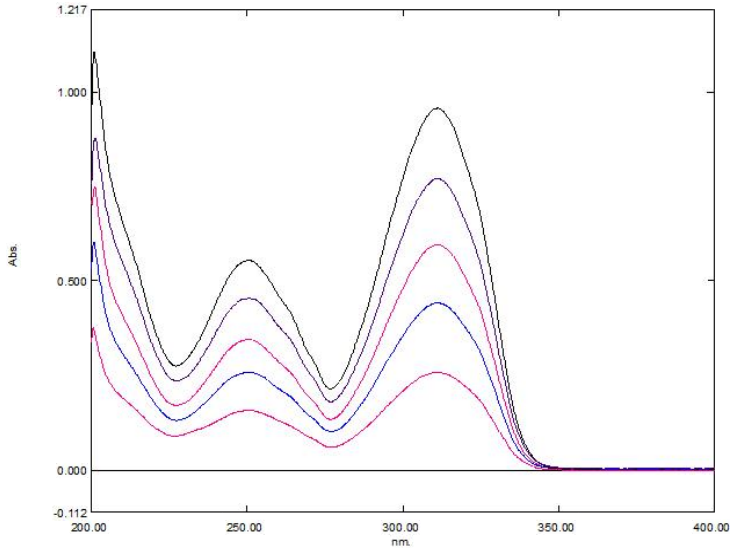

Figure 2: UV Zero-Order Derivative Calibration Curve of Abametapir (2–10 µg/mL) at λmax 311 nm

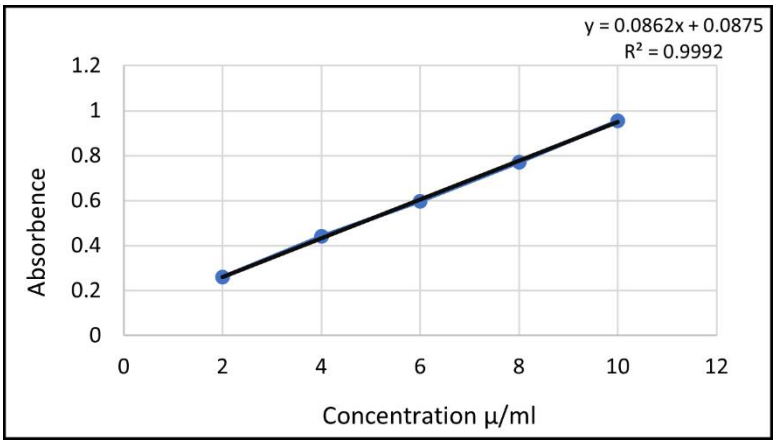

Figure 3: Calibration curve of Abametapir at 311 nm.

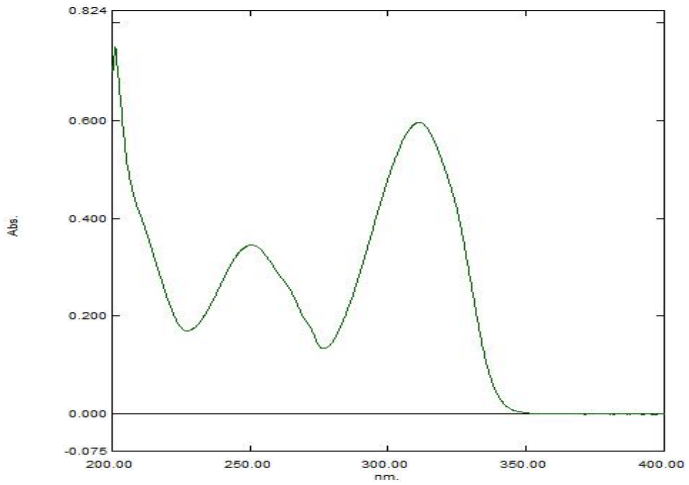

Figure 4: UV Zero-Order Derivative Curve of Abametapir 6 µg/mL

2.7 Linearity and Range

The linearity of the zero-order derivative spectrophotometric method for Abametapir was evaluated over a concentration range of 2–10 µg/mL. Calibration curves were constructed by plotting the derivative response at 311 nm against the corresponding concentrations. The method was assessed in triplicate for each level, and linear regression analysis yielded a slope of 0.0862, an intercept of 0.0875, and an excellent correlation coefficient (R^2 = 0.9992), indicating a strong linear relationship. The calculated molar absorptivity was 0.106187 $L \cdot mol^{-1} \cdot cm^{-1}$.

Repeatability was confirmed by %RSD values ranging from 0.16% to 0.97%, consistent with ICH Q2(R1) guidelines. The method also demonstrated high sensitivity, with LOD and LOQ values of 0.10962 µg/mL and 0.33218 µg/mL, respectively, confirming its suitability for precise quantitative analysis.

2.8 Accuracy

The accuracy of the zero-order derivative spectrophotometric method for Abametapir was evaluated using the standard addition technique at three concentration levels: 50%, 100%, and 150% of the target concentration (4 µg/mL). Known quantities of standard Abametapir were added to pre-analyzed samples, and each level was analyzed in triplicate. Statistical parameters—including percentage recovery, standard deviation (SD), coefficient of variation (%CV), and standard error (SE)—were calculated to assess the trueness and reproducibility of the method.

The mean percentage recovery ranged from 98.72% to 99.88%, confirming the method's accuracy and reliability in accordance with ICH Q2(R1) guidelines.

2.9 Precision

Precision of the zero-order derivative spectrophotometric method was evaluated by assessing both repeatability (intra-day) and intermediate precision (inter-day) using replicate analyses of a 6 µg/mL Abametapir solution. Intra-day precision was assessed on the same day, while inter-day precision was performed across three consecutive days.

The method demonstrated robust reproducibility, as evidenced by mean percentage recoveries of 99.53% (intra-day) and 99.82% (inter-day). Standard deviation, coefficient of variation (%CV), and standard error values were within acceptable limits, with %CV values ≤2%, confirming the reliability of the procedure as per ICH Q2(R1) guidelines.

2.10 Intraday Precision

Intraday precision of the zero-order derivative spectrophotometric method was evaluated through six replicate determinations of Abametapir (6 µg/mL) conducted on the same day under consistent experimental conditions. Percentage recovery was calculated for each replicate, and statistical parameters—including mean, standard deviation (SD), and coefficient of variation (%CV)—were employed to assess repeatability.

The method demonstrated excellent intraday precision, with %CV values well below 2%, confirming its reproducibility and compliance with ICH Q2(R1) guidelines.

2.11 Interday Precision

The interday precision of the zero-order derivative spectrophotometric method was determined by evaluating Abametapir (6 µg/mL) over three consecutive days. Six replicate determinations were performed on each day under identical experimental conditions. The results were analyzed statistically to compute mean percentage recovery, standard deviation (SD), and coefficient of variation (%CV), thereby establishing the intermediate precision of the method across days.

Consistently low %CV values (≤2%) and high mean recoveries confirmed the reliability and robustness of the method under variable temporal conditions, in accordance with ICH Q2(R1) guidelines.

2.12 Specificity

Specificity of the zero-order derivative spectrophotometric method was established by comparing the derivative response of the Abametapir standard solution (6 µg/mL) with a blank prepared using 0.1 N HCl. No interfering signals were observed at the selected wavelength (311 nm), confirming that the method specifically detects Abametapir in its bulk form without interference from excipients or reagents.

These findings affirm the method's selectivity and compliance with the specificity criteria outlined in ICH Q2(R1) guidelines.

Forced Degradation Studies:

Forced degradation was performed in accordance with ICH Q1A(R2) guidelines. For each condition, 50 mg of Abametapir was used to prepare Stock A (1000 µg/mL), followed by dilution to Stock B (10 µg/mL) and preparation of working solutions (2–10 µg/mL):

1. ***Acidic Hydrolysis***

- Refluxed in 0.1 N HCl at 80 °C for 3 hours
- Post-reflux sample diluted in 0.1 N HCl and scanned at 311 nm

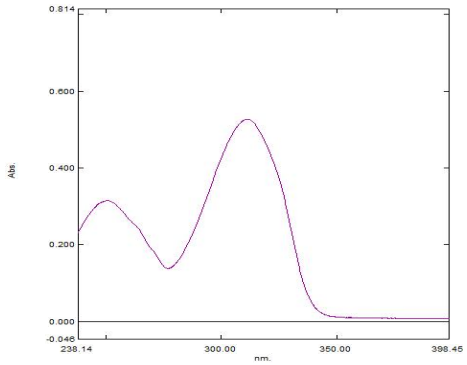

Figure 5: UV Spectrum of Abametapir After Acidic Hydrolysis

2. Alkaline Hydrolysis

- Refluxed in 0.1 N NaOH at 80 °C for 3 hours
- Post-reflux sample neutralized, diluted in 0.1 N HCl, and scanned

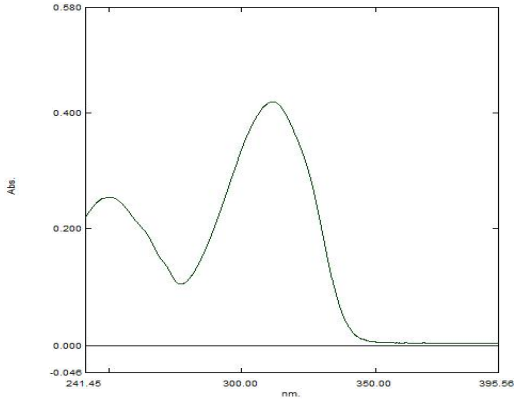

Figure 6: UV Spectrum of Abametapir After Alkaline Hydrolysis

3. Oxidative Stress

- Treated with 3% H_2O_2 at 80 °C for 3 hours under reflux
- Resulting sample diluted in 0.1 N HCl and scanned

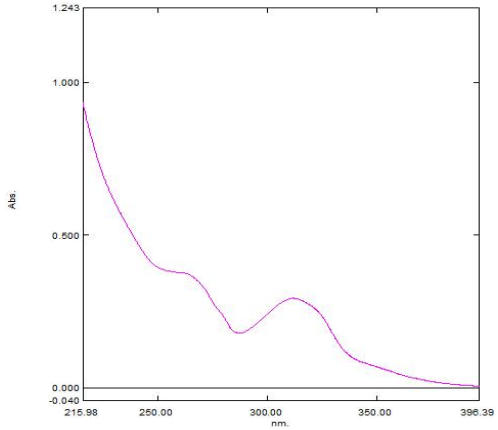

Figure 7: UV Spectrum of Abametapir After Oxidative Stress

4. Photolytic Stress

- 50 mg spread on a glass plate and exposed to direct sunlight for 12 hours
- Exposed sample then diluted in 0.1 N HCl

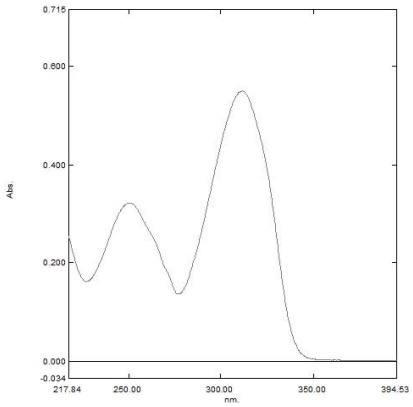

Figure 8: UV Spectrum of Abametapir After Photolytic Stress

5. Thermal Stress

- Solid sample placed in hot air oven at 80 °C for 3 hours
- Cooled and diluted in 0.1 N HCl

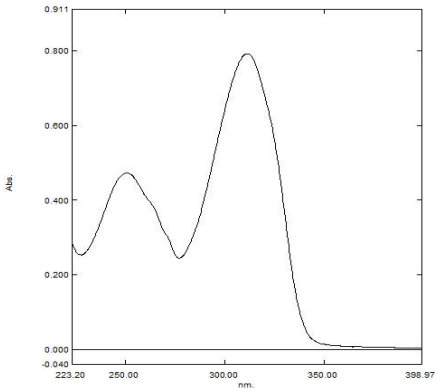

Figure 9: UV Spectrum of Abametapir After Thermal Stress

4. Results

4.1 Determination and Calibration Curve

Abametapir exhibited a sharp absorbance peak at 311 nm, which was selected as the analytical wavelength for all measurements. A calibration curve was constructed using zero-order derivative spectrophotometric analysis over the concentration range of 2–10 µg/mL, yielding the regression equation $Y = 0.0862x + 0.0875$ with a correlation coefficient (R^2) of 0.9992, confirming excellent linearity. The molar absorptivity was calculated as 0.106187 $L \cdot mol^{-1} \cdot cm^{-1}$.

Table 1: Calibration Curve Data for Abametapir at 311 nm.

Concentration (µg/mL)	Mean Absorbance ± SD	% RSD
2	0.259 ± 0.00252	0.97%
4	0.441 ± 0.00351	0.80%
6	0.596 ± 0.00252	0.42%
8	0.770 ± 0.00321	0.42%
10	0.956 ± 0.00153	0.16%

4.2 Linearity and Range

All four methods showed excellent linearity in the concentration range of 2–10 µg/mL, with correlation coefficients (R^2) consistently greater than 0.999. The slope, intercept, and regression data confirmed a strong linear response, indicating the methods' suitability for routine analysis of Abametapir.

Table 2: Linearity and Range Parameters for Zero-Order Derivative Method

Sl. No	Parameter	Zero-order derivative
1	λ_{max} (nm)	311 nm
2	Linearity range (µg/mL)	2-10 µg/mL
3	Molar absorptivity (liter, mole^{-1} cm^{-1})	0.106187
4	Slope (m)	0.0862
5	Intercept(c)	0.0875
6	Correlation coefficient (R^2)	0.9992
7	Range of % RSD	0.16% - 0.97%
8	LOD(µg/mL)	0.10962
9	LOQ (µg/mL)	0.33218

4.3 Accuracy

Accuracy was evaluated by standard addition at 50%, 100%, and 150% levels of a 4 µg/mL target concentration. The mean recovery ranged from 98.72% to 99.88%, confirming high accuracy. Statistical validation showed low standard errors.

Table 3: Accuracy – Recovery Study Data of Abametapir.

Percentage Recovery Level	Amount Present (µg/mL)	Standard Added (µg/mL)	Total Recovery (µg/mL)	Percentage Recovery
50%	4	2	5.92	98.67
	4	2	5.89	98.17
	4	2	5.96	99.33
100%	4	4	8.10	101.25
	4	4	7.95	99.38
	4	4	7.92	99.00
150%	4	6	9.82	98.20
	4	6	10.16	101.60
	4	6	9.88	98.80

Table 4: Statistical Validation of Accuracy of Abametapir.

% Recovery Level	Mean Recovery (%)	Standard Deviation (SD)	Coefficient of Variation (CV)%	Standard Error (SE)
50%	98.72	0.585	0.593	0.338
100%	99.88	1.205	1.207	0.696
150%	99.53	1.815	1.823	1.048

4.4 Precision

Precision studies demonstrated excellent method reproducibility.

- **Intra-day precision** showed a mean % recovery of 99.53% with a %RSD of 0.94%.
- **Inter-day precision** across three consecutive days showed a pooled mean % recovery of 99.82% and an overall %RSD of 0.23%, confirming intermediate precision and consistency of results over time.

Table 5: Precision Results of Abametapir.

Validation Parameter	Mean Recovery (%)	Standard Deviation	Coefficient of Variation (%)	Standard Error
Intra-day Precision	99.53	0.980	0.985	0.400
Inter-day Precision	99.82	0.226	0.227	0.053

4.5 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The sensitivity of the proposed method was evident from its low limit of detection (LOD) and quantification (LOQ), as summarized in Table [2], thereby underscoring its suitability for precise estimation of Abametapir even at low concentration levels.

4.6 Specificity

Specificity was confirmed by comparing the UV spectrum of the Abametapir standard solution with that of the blank (0.1 N HCl). No absorbance or interference was observed at the selected wavelength, indicating that the method can accurately measure Abametapir without any overlapping signals from the blank or other components.

4.7 Interpretation of Degradation.

Forced degradation outcomes under ICH Q1A(R2)

- The highest degradation was observed under oxidative stress (50.67%), attributed to radical-mediated cleavage and possible peroxide-induced oxidation of aromatic centers.
- Alkaline conditions led to notable degradation (29.87%), indicating sensitivity to nucleophilic attack or hydroxide-catalyzed hydrolysis.
- Acidic hydrolysis showed a moderate effect (11.56%) with visible peak dampening.
- Both photolytic and thermal stress revealed minimal degradation (<8%), suggesting overall photostability and thermal tolerance within analytical limits.

Table 6. Forced Degradation Profile of Abametapir (6 µg/mL, measured at 311 nm)

Stress Condition	Medium Used	Absorbance	Percentage Recovery	Percentage Degradation
Zero order	0.1 N HCl	0.596	100.00%	0.00%
Acidic Hydrolysis	0.1 N HCl	0.527	88.44%	11.56%
Alkaline Hydrolysis	0.1 N NaOH	0.418	70.13%	29.87%
Oxidative Stress	3% H_2O_2	0.294	49.33%	50.67%
Thermal Stress	Dry heat at 80 °C	0.559	93.79%	6.21%
Photolytic Stress	Sunlight (12 hrs)	0.549	92.12%	7.88%

5. Discussion

The forced degradation study of Abametapir using a zero-order derivative UV spectrophotometric method revealed insightful trends regarding the drug's chemical stability. Among the five stress conditions applied, oxidative and alkaline environments demonstrated significant degradation, suggesting susceptibility of Abametapir to base-catalyzed hydrolysis and oxidative cleavage. The high degradation observed under oxidative stress (50.67%) may indicate instability in the presence of peroxides or other reactive oxygen species, potentially involving aromatic ring oxidation or disruption of labile functional groups.

Alkaline hydrolysis resulted in 29.87% degradation, consistent with the presence of base-labile moieties, whereas acidic hydrolysis yielded only 11.56%, suggesting comparative stability under proton-rich environments. Thermal (6.21%) and photolytic (7.88%) stresses caused minimal degradation, implying relative structural robustness under heat and light exposure.

Calibration results confirmed excellent linearity across 2–10 µg/mL, with $R^2 = 0.9992$, and low %RSD values across all replicates, validating method precision and reproducibility. Sensitivity, reflected in low LOD and LOQ values (0.10962 µg/mL and 0.33218 µg/mL, respectively), ensures accurate

detection of even minor degradation. Recovery studies across varying levels (50%, 100%, 150%) further support the method's analytical validity, with recoveries consistently between 98.16% and 101.6%.

These findings demonstrate the method's applicability as a stability-indicating tool for Abametapir. The selective sensitivity to degradation and precise quantification enable its use in both preformulation screening and routine quality control, especially when rapid, non-chromatographic techniques are preferred.

6. Conclusion

The developed zero-order derivative UV spectrophotometric method provides a reliable and cost-effective approach for assessing the stability of Abametapir. Spectral measurements at 311 nm enabled clear differentiation between intact and degraded drug profiles across ICH-recommended stress conditions. The method demonstrated excellent linearity ($R^2 = 0.9992$), precision (%RSD < 2%), and sensitivity (LOD: 0.10962 µg/mL, LOQ: 0.33218 µg/mL), validating its suitability for quantitative degradation analysis. Among the applied stressors, oxidative and alkaline environments induced the highest levels of degradation (50.67% and 29.87%, respectively), highlighting specific vulnerabilities in the drug's structure. Minimal changes under acidic, thermal, and photolytic stress further confirm its partial stability. Overall, the study establishes a robust, reproducible method for monitoring Abametapir degradation—ideal for preformulation assessment and routine quality control in pharmaceutical research.

conflict of interest

The author discloses no conflicts of interest regarding the development, execution, or publication of this research work.

List of abbreviations

API—Active Pharmaceutical Ingredient, UV—Ultraviolet, nm—Nanometer, SD—Standard Deviation, %RSD—Percent Coefficient of Variation/Relative Standard Deviation, SE—Standard Error, ICH—International Council for Harmonization, AUC—Area Under the Curve, λmax—Wavelength of Maximum Absorbance, HCl—Hydrochloric Acid, µg/mL—Micrograms per Milliliter.

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