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Gokulapriya A
M. Pharm student,
Department of Pharmaceutical
Analysis, Adhiparasakthi
College of Pharmacy,
Melmaruvathur, Affiliated to
The Tamil Nadu Dr. M. G. R.
Medical University,
Chennai/Tamil Nadu, India

Vetrichelvan T
HOD cum Dean Research,
Department of Pharmaceutical
Analysis, Adhiparasakthi
College of Pharmacy,
Melmaruvathur, Affiliated to
The Tamil Nadu Dr. M. G. R.
Medical University,
Chennai/Tamil Nadu, India

Murugan S
Associate Professor,
Department of Pharmaceutical
Analysis, Adhiparasakthi
College of Pharmacy,
Melmaruvathur, Affiliated to
the Tamil Nadu Dr. M. G. R.
Medical University,
Chennai/Tamil Nadu, India

Correspondence
Gokulapriya A
M. Pharm student,
Department of Pharmaceutical
Analysis, Adhiparasakthi
College of Pharmacy,
Melmaruvathur, Affiliated to
The Tamil Nadu Dr. M. G. R.
Medical University,
Chennai/Tamil Nadu, India

Forced degradation studies, method development and validation of UV spectrophotometric method for the analysis of endoxifen citrate in bulk and tablet dosage form

Gokulapriya A, Vetrichelvan T and Murugan S

Abstract

Novelty: Endoxifen citrate was quantified utilizing zero-order and first-order derivative approaches in a straightforward, quick, and affordable UV spectrophotometric method. To the best of our knowledge, no such analytical method has been previously reported for the estimation of this drug in bulk and tablet dosage form.

Objective: To develop and validate a novel UV spectrophotometric method for Endoxifen citrate quantification as per ICH guidelines.

Method: Distilled water was used as the solvent for the analysis, and the absorbance was maximum at 227 nm (zero order) and 211 nm (first order). Linearity was observed between 24-72 µg/mL. Forced degradation was studied under acidic, basic, oxidative, reductive, and thermal conditions.

Results: Excellent linearity ($r^2 = 0.9997$ and 0.9999), accuracy (98.12-100.39 %), and precision (%RSD < 1.5 %) were achieved. The drug degraded only under acidic conditions.

Conclusion: The suggested approach is appropriate for routine estimation of endoxifen citrate in pharmaceutical formulations since it is straightforward, accurate, exact, and cost-effective.

Keywords: Endoxifen citrate, derivative spectroscopy, zero order spectra, first order derivative, analytical method validation

Introduction

Endoxifen was discovered in 1988 as a metabolite of tamoxifen. Tamoxifen is a type of selective estrogen receptor modulator (SERM), meaning it can act like estrogen in some tissues while blocking its effects in others. In breast tissue, tamoxifen primarily exhibits antiestrogenic activity. It was initially developed as an oral contraceptive but proved ineffective for that purpose. However, during the 1960s and 1970s, its anticancer properties were identified. Since blocking estrogen receptors can inhibit tumor growth, tamoxifen was developed as a treatment for women with estrogen receptor-positive breast cancer.

The chemical structure of Endoxifen is identified as 4-[(E)-1-[4-[2-(methylamino) ethoxy] phenyl]-2-phenylbut-1-enyl] phenol citrate. Although its chemical identity has been recognized for several years, Pharmaceutical industries initially expressed reservations regarding its development due to limited intellectual property protection. To address this, a research team at the Mayo Clinic, in collaboration with the National Cancer Institute (NCI), initiated efforts to establish a public-private partnership aimed at evaluating the antitumor potential of Endoxifen and assessing its viability as a therapeutic drug candidate^[1, 2].

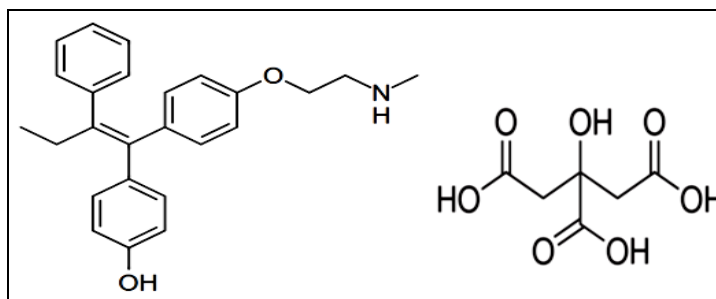


Fig 1: Chemical structure of Endoxifen citrate^[3]

A review of the existing literature revealed that no analytical method has been reported for the estimation of Endoxifen in bulk and tablet formulations using zero-order and first-order derivative spectrophotometry. Therefore, the objective of the present study was to develop a novel, simple, precise, and economical UV spectrophotometric method for the assay of Endoxifen in bulk and tablet dosage form in accordance with ICH guidelines. The developed method was subsequently validated as per ICH requirements^[4, 5].

2. Materials and Methods

Instrumentation

The instrument employed in the present study was a Shimadzu double-beam UV-Visible spectrophotometer (Model UV-1700) with a spectral bandwidth of 1 nm. All weighing procedures were performed using an electronic analytical balance (Model Shimadzu AUX-220).

Reagents and chemicals

All chemicals and reagents of HPLC grade were procured from Qualigens India Pvt. Ltd., Mumbai, India.

The pharmaceutical dosage form utilized in this trial was the ZONALTA Tablet, which included 8 mg of endoxifen. It was acquired from Apollo Pharmacy in Chennai, and API Pharmaceuticals provided the endoxifen citrate API.

Analytical method development for zero order and first order derivative

Preparation of Standard stock solution

An accurately weighed quantity of Endoxifen equivalent to **60 mg** was transferred into a 10 mL volumetric flask, dissolved in a small volume of distilled water, and the volume was made up to the mark with the same solvent to obtain a stock solution of 6000 µg/mL.

Preparation of working standard solution

600 µg/mL of Endoxifen working standard solution was prepared by diluting 1 mL of standard stock solution with water in 10 mL volumetric flask up to the mark.

Selection of wavelength for measurement

A solution containing 10 µg/mL of Endoxifen was obtained by diluting 0.6 mL of the working standard solution (36 µg/mL) with 10 mL of water. This solution was scanned against water at 200-400 nm. Endoxifen clearly exhibits an absorption at 211 nm for first order and at 227 nm for zero order. "Figs. 2 and 3" illustrates it.

Preparations of sample solution

Twenty tablets of the formulation ZONALTA®, each containing 8 mg of Endoxifen, were accurately weighed to determine the average tablet weight and subsequently powdered. A portion of the powdered tablets equivalent to 6 mg of Endoxifen was transferred to a 10 mL volumetric flask, dissolved in a minimal volume of distilled water using a sonicator for 15 minutes, and the volume was adjusted to the mark with distilled water to obtain the sample stock solution. The solution was filtered through Whatman filter paper No. 41. From the filtrate, 0.6 mL was further diluted to 10 mL with distilled water to prepare a 36 µg/mL solution. The concentration of Endoxifen was then determined using the zero-order method at 227 nm and the first-order derivative method at 211 nm. This procedure was repeated six times for each concentration to ensure

reproducibility.

3. Method Validation for Zero Order and First Order Linearity

Aliquots of the working solution of Endoxifen (**600 µg/mL**) with volumes of 0.4, 0.6, 0.8, 1.0, and 1.2 mL were transferred into 10 mL volumetric flasks. The volume in each flask was adjusted to the mark with distilled water to obtain final concentrations of 24, 36, 48, 60, and 72 µg/mL, respectively. The absorbance of each solution was measured at 227 nm for the zero-order spectrum and at 211 nm for the first-order derivative spectrum, using distilled water as the blank. Calibration curves of absorbance versus concentration were plotted, as shown in Figures 4 and 5.

Precision

(a) Repeatability

A solution was prepared by pipetting 0.6 mL of the working standard solution (600 µg/mL) into a 10 mL volumetric flask, and the volume was adjusted to the mark with distilled water. This solution (36 µg/mL) was determined for 6 times. Measure the solution at 227 nm for zero order and for first order at 211 nm and calculate % RSD.

(b) Precision within the day (Intraday)

Analysing the solution (36 µg/mL) six times on the same day allowed for the determination of intra-day precision. The findings were presented as a percentage RSD.

(c) Precision between days (Interday)

By analysing the solution (36 µg/mL) six times on various days, the inter-day precision was ascertained. The findings were presented as a percentage RSD.

Accuracy

By using the conventional addition method to calculate the recovery of Endoxifen, accuracy was ascertained. A prequalified test solution of Endoxifen (36 µg/mL) was mixed with known concentrations of reference solutions (14.4, 28.8, 43.2 µg/mL). The recovery was determined by measuring absorbance, and each solution was measured three times.

Ruggedness

The ruggedness of the proposed method was evaluated by analysing aliquots from a homogeneous sample by two different analysts under varying operational and environmental conditions.

Limit of Detection and Limit of Quantitation

They were measured using standard deviation of Y-intercept and slope of calibration curve as per ICH guideline. The LOD and LOQ were calculated using following formula

$$\begin{aligned}\text{LOD} &= 3.3 \times \sigma / s \\ \text{LOQ} &= 10 \times \sigma / s\end{aligned}$$

Where,

σ = the standard deviation of response

s = the slope of the calibration curve

Forced Degradation studies for zero order method:^{[8][9][10]}

Endoxifen API was undergone forced degradation study

under acidic and basic degradation as well as Thermal degradation, oxidation, reduction, stress conditions.

Acid degradation

In order to conduct acid degradation studies, 0.6 mL of the working standard solution was transferred into a 10 mL volumetric flask. After adding and thoroughly mixing 0.6 mL of 0.1 N H_2SO_4 solutions, the mixture was left for a full day. To neutralize the solution and replenish the volume with diluent, 2 mL of 0.1 N KOH was added after the time interval.

Base degradation

In order to conduct base degradation investigations, 0.6 mL of the working standard solution was transferred into a 10 mL volumetric flask. After adding and thoroughly mixing 0.6 mL of 0.1 N KOH solutions, the mixture was left for a full day. To neutralize the solution and replenish the volume with diluent, 2 mL of 0.1 N H_2SO_4 was added after the time interval.

Oxidation degradation

In order to conduct oxidation degradation investigations, 0.6 mL of the working standard solution was transferred into a 10 mL volumetric flask. After adding and thoroughly mixing 0.6 mL of 1% H_2O_2 solutions, the mixture was left for a full day. After a while, use diluent to adjust the volume.

Reduction degradation: In order to conduct reduction

degradation investigations, 0.6 mL of the working standard solution was transferred into a 10 mL volumetric flask. After adding and thoroughly mixing 0.6 mL of 10% sodium bisulfate solutions, the mixture was left for a full day. After a while, use diluent to adjust the volume.

Thermal degradation

To conduct thermal degradation investigations, a precisely weighed amount of Endoxifen, equal to 80 mg, was transferred into a 25 mL volumetric flask that had been previously stored in a hot air oven at 70°C for 24 hours. The volume was then adjusted with distilled water as a diluent. 2.5 mL of the above-mentioned solution was diluted and the volume was adjusted with diluent in a 50 mL volumetric flask. 2 mL of the above-mentioned solution was pipetted into a 10 mL volumetric flask, and the remaining volume was filled with diluent^[7].

4. Results and Discussion

The International Council for Harmonization (ICH) guidelines were followed in the validation of the developed UV spectrophotometric methods (zero-order and first-order derivative) for Endoxifen. The parameters that were evaluated included linearity, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ), and ruggedness.

The UV spectra for Endoxifen are shown in Figure 2 (zero-order, $\lambda_{\text{max}} = 227 \text{ nm}$) and Figure 3 (first-order derivative, $\lambda_{\text{max}} = 211 \text{ nm}$).

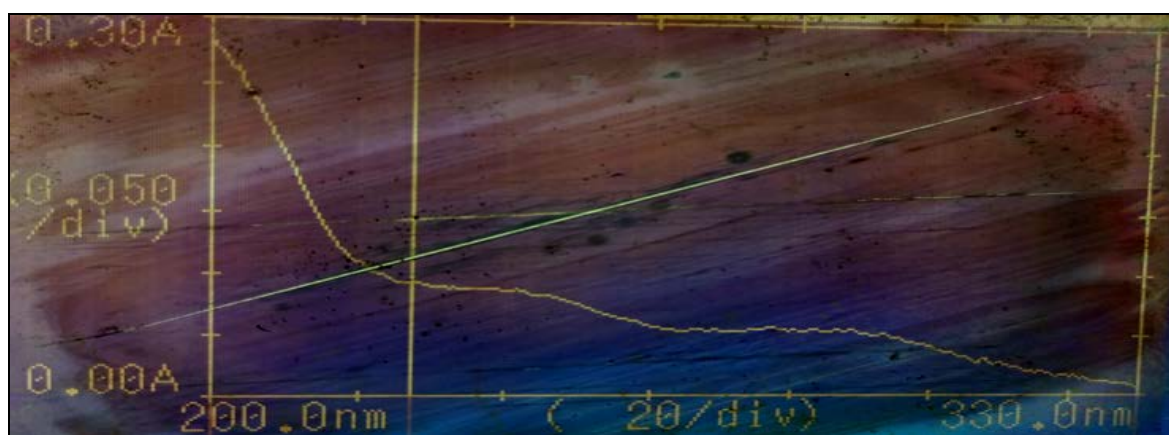


Fig 2: Zero order spectrum of Endoxifen

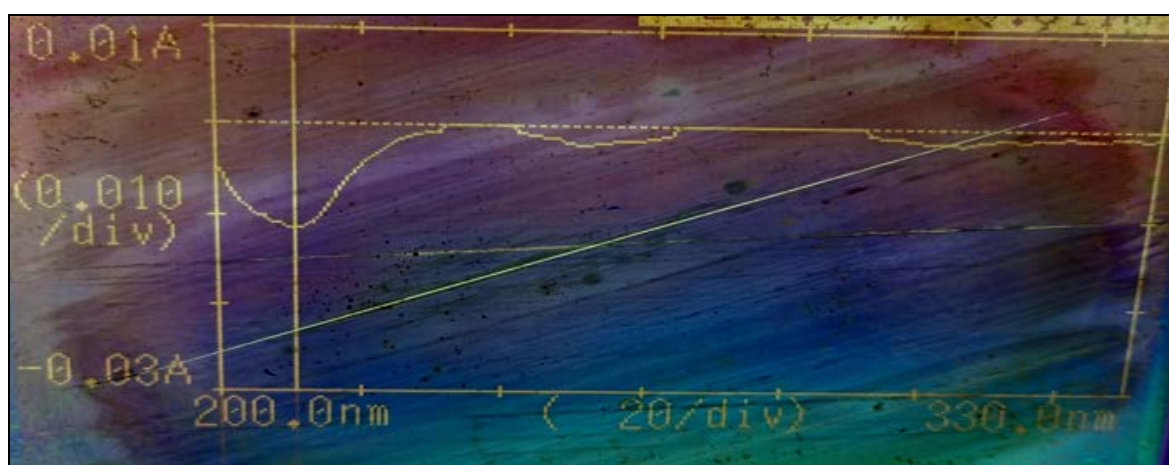


Fig 3: First order spectra of Endoxifen

Linearity

By measuring absorbance at 227 nm for zero-order derivative methods and 211 nm for first-order derivative methods, calibration curves were created throughout a concentration range of 9-45 µg/mL (Figures 4 and 5). Table

1 lists the optical parameters, such as the correlation coefficient, slope, intercept, LOD, and LOQ. Excellent linearity was indicated by the correlation coefficients, which were 0.9997 (zero-order) and 0.9999 (first-order).

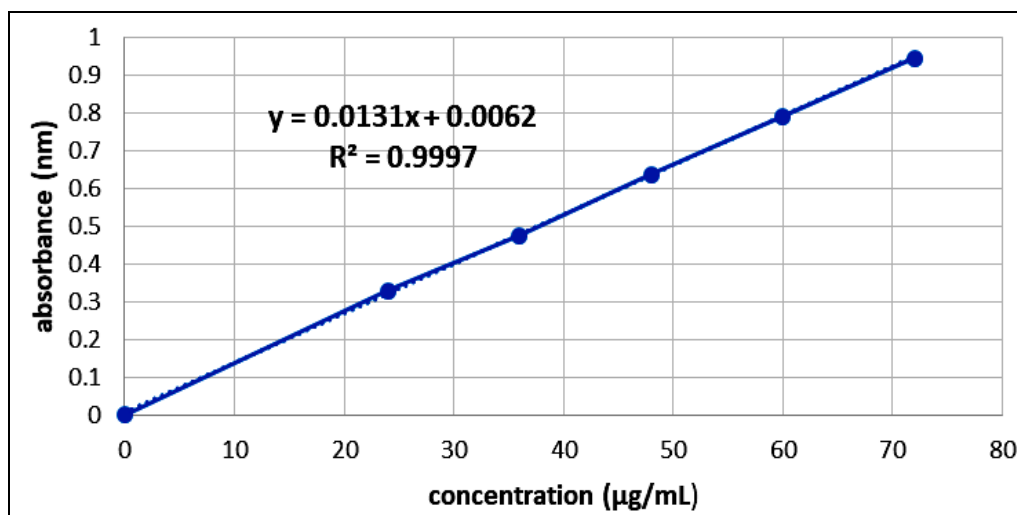


Fig 4: Calibration graph of Endoxifen for zero order

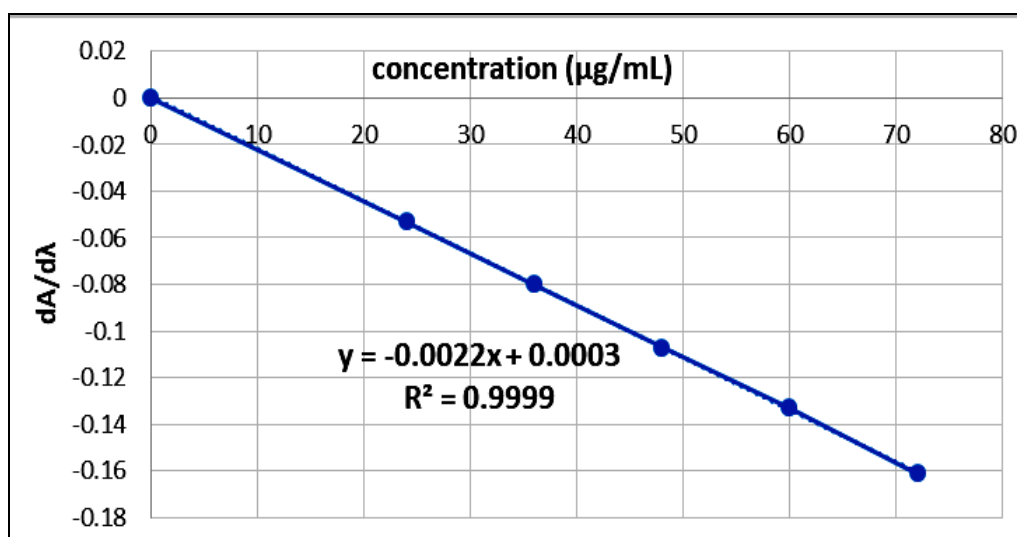


Fig 5: Calibration graph of Endoxifen for first order

Table 1: Optical parameters and linearity data of Endoxifen

Parameters	Zero order method (227 nm)	First order derivative (211 nm)
Beer's law limit(µg/mL)	24-72	24-72
Regression equation ($y = mx+c$)	$y = 0.0131x+0.0062$	$y = -0.0022x+0.0003$
Slope (m)	0.0131	-0.0022
Intercept (c)	0.0062	0.0003
Correlation co-efficient (R^2)	0.9997	0.9999
SE of intercept	0.0051	0.0003
S. D	0.0124	0.0009
LOD (µg/mL)	3.148	1.4193
LOQ (µg/mL)	9.5417	4.3011

Precision

Endoxifen citrate tablets containing 8 mg of Endoxifen were assayed using both methods. The results, summarized in Table 2, showed mean assay values of 99.58 ± 0.3042 for

the zero-order method and 100.53 ± 1.0152 % for the first-order method. The %RSD values were below 2%, confirming good precision in the assay.

Table 2: Precision of Endoxifen Formulation

Sample	Methods	Sample number	Label claim (mg/tab)	Amount present (mg/tab)	% purity	Mean purity	SD	% RSD
END	Zero order	1	8	7.95	99.37%	99.58	0.3030	0.3042
		2	8	7.97	99.62%			
		3	8	8.00	100%			
		4	8	7.95	99.37%			
		5	8	7.99	99.87%			
		6	8	7.94	99.25%			
	First order derivative	1	8	8.01	100.12	100.53	1.0206	1.0152
		2	8	8.11	101.37			
		3	8	7.91	98.87			
		4	8	8.11	101.37			
		5	8	8.01	100.12			
		6	8	8.11	101.37			

Intraday and Interday precision

Precision tests were conducted both within and between days at a nominal concentration of 36 µg/mL. The percentage RSD values for the zero-order were 0.256 (inter-

day) and 0.323 (intra-day), but the first-order values were 0.7797 (inter-day) and 1.1116 (intra-day). High technique precision was indicated by all data falling below the 2% limit. The findings are displayed in Table 3.

Table 3: Intraday and Interday of Endoxifen Formulation

Sample	Methods	Sample number	Label claim (mg/tab)	% purity		SD *		%RSD *	
				Intra day	Inter Day	Intra day	Inter day	Intra Day	Inter Day
END	Zero order	1	8	99.50 99.37 99.75 99.25 99.50 99.25	99.62 99.87 99.25 100 99.87 99.62	0.322	0.255	0.323	0.256
		2	8						
		3	8						
		4	8						
		5	8						
		6	8						
		Mean		99.56	99.45				
	First order derivative	1	8	99 98.87 101 101.37 99.83 100.12	100 101.37 99.37 98.87 100.87 100.12	1.318	0.983	1.1116	0.7797
		2	8						
		3	8						
		4	8						
		5	8						
		6	8						
		Mean		100.12	100.01				

Accuracy

The conventional addition method was used to assess accuracy at three different concentration levels. The range of recoveries for the zero-order approach was 100.27

percent to 100.39 percent, while for the first-order method, it was 98.12 percent to 100.04 percent. Low percentage RSD values attested to both approaches' certainty. Table 4 presents the findings.

Table 4: Recovery Study

Sample	Methods	%	Sample amount (µg/ml)	Amount spiked (µg/ml)	Estimated Amount * (µg/ml)	Recovered Amount * (µg/ml)	Average* % Recovery	SD	%RSD
END	Zero order	40	36	14.4	50.44	14.44	100.27	1.0400	1.0300
		80	36	28.8	64.79	28.79	99.96	0.2800	0.2801
		120	36	43.2	79.29	43.29	100.39	0.1752	0.1740
	First order derivative	40	36	14.4	50.13	14.13	98.12	1.8475	1.8828
		80	36	28.8	64.68	28.8	99.58	1.5661	1.5727
		120	36	43.2	79.22	43.22	100.04	1.0500	1.0495

* Mean of 3 Observations

Ruggedness

Ruggedness was evaluated using two different analysts and two instruments. For the zero-order method, the %RSD values were 0.3147 and 0.2780 (instrument variation) and 0.3866 and 0.3158 (analyst variation). For the first-order

method, the values were 0.5587 and 0.7344 (instrument variation) and 1.0865 and 0.6502 (analyst variation). All %RSD values were below 2%, indicating that both methods are rugged. Table 5 presents the findings.

Table 5: Ruggedness Study of Endoxifen Formulation (Instrumental and analyst variation)

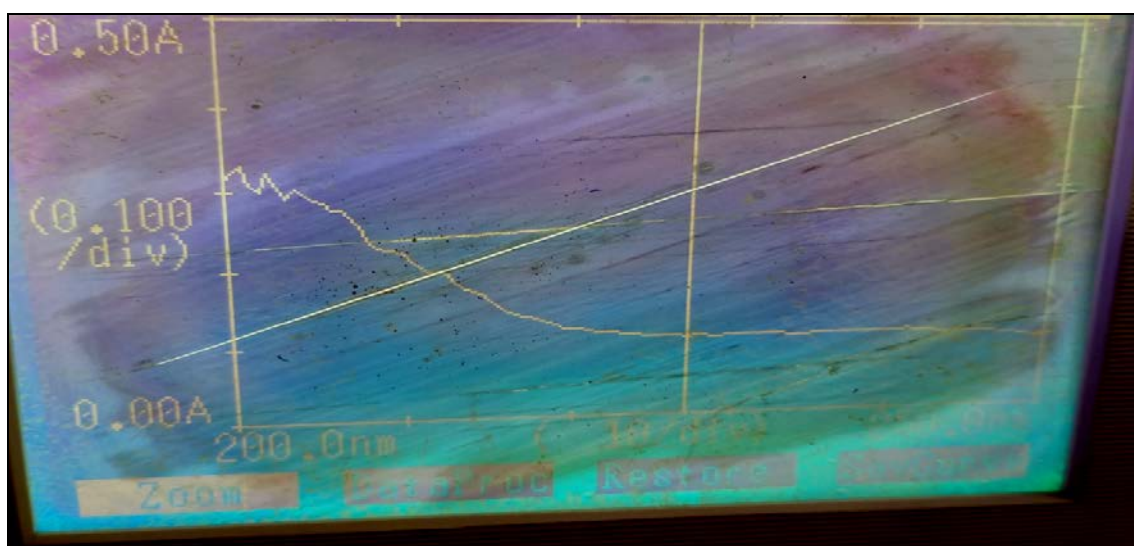
Sample	Methods	Type of ruggedness	Average % obtained *	SD	% RSD
END	Zero order	Analyst 1	99.58	0.3850	0.3866
		Analyst 2	99.71	0.3149	0.3158
		Instrument 1	99.89	0.3144	0.3147
		Instrument 2	100.03	0.2781	0.2780
	First order derivative	Analyst 1	100.25	1.0893	1.0865
		Analyst 2	100.02	0.6504	0.6502
		Instrument 1	100.57	0.5619	0.5587
		Instrument 2	100.23	0.7361	0.7344

* Mean of 6 Observations

Forced degradation studies for the zero-order method

Endoxifen was subjected to forced degradation under acidic, alkaline, oxidative, reductive and thermal stress conditions. Significant degradation was observed only under acidic conditions, with 77.45 % degradation, respectively (Figures 6), while the drug remained stable under all other conditions. These results indicate that Endoxifen is

susceptible to acid-induced degradation but stable under alkaline, oxidative, reductive and thermal stress. The developed zero-order UV spectrophotometric method successfully detected these changes, confirming its suitability for routine stability studies of bulk drug and tablet formulation.

**Fig 6:** Acid degradation spectra of Endoxifen**Table 6:** Endoxifen Forced degradation studies results in different parameters

Stress condition	Time(h)	% degradation
0.1M H ₂ SO ₄	24	77.45
0.1M KOH	24	Not degraded
Oxidation	24	Not degraded
Reduction	24	Not degraded
Thermal	24	Not degraded

Conclusion

The current work effectively created and verified a straightforward, accurate, and reasonably priced UV spectrophotometric technique for the quantitative measurement of endoxifen citrate in tablet and bulk dose forms. The results showed adequate linearity, precision, accuracy, and robustness, and the method was validated in compliance with ICH requirements.

Studies on forced deterioration validated the method's stability-indicating character. Under acidic conditions, endoxifen degrades significantly, whereas under alkaline, oxidative, reduction, and heat environments, they degraded relatively little. The findings show that endoxifen citrate is especially vulnerable to hydrolysis brought on by acids.

All things considered, the suggested UV

spectrophotometric approach is trustworthy for both stability testing during formulation development and routine quality control analysis of endoxifen citrate in pharmaceutical formulations.

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Authors Contributions

Each author has made an equal contribution.

Conflict of Interests

Proclaimed none.

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