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Analytical method development and validation of stability indicating UV spectrophotometric method for pitolisant Hydrochloride in bulk and tablet dosage form

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Abstract
Objective: The present study aimed to develop and validate a simple, cost-effective, and reliable UV-spectrophotometric method for the quantification of Pitolisant Hydrochloride in bulk and tablet formulations, in accordance with ICH guidelines.
Methods: Quantification was performed using a UV-spectrophotometer with distilled water as solvent. Pitolisant showed maximum absorbance at 221 nm (zero order) and 228 nm (first order). The linearity range was 22.25-111.25 µg/ml, with correlation coefficients (r^2) of 0.9997 and 0.9998 for zero and first order, respectively.
Results: All validation parameters linearity, accuracy, precision, LOD, LOQ, and recovery were within acceptable limits, confirming the reliability of the developed methods for both zero and first order derivatives.
Conclusion: The developed UV-spectrophotometric techniques provide a straightforward, reliable, and cost-effective approach for the routine quantitative determination of Pitolisant Hydrochloride in tablet formulations.

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

Introduction

Pitolisant is a selective antagonist and inverse agonist of the histamine H3 receptor, representing an innovative therapeutic option for managing narcolepsy and other disorders characterized by excessive daytime sleepiness. Its pharmacological action is mediated through the enhancement of histaminergic neurotransmission within the central nervous system, thereby promoting wakefulness and mitigating sleep attacks. Chemically, Pitolisant is identified as 1-[3-[3-(4-chlorophenyl) propoxy] propyl] piperidine hydrochloride, with the molecular formula C17H27Cl2NO·HCl and a molecular weight of 332.3 g/mol.

This compound exhibits high selectivity for H3 receptors relative to other histamine receptor subtypes. Additionally, Pitolisant modulates the release of key neurotransmitters such as acetylcholine, noradrenaline, and dopamine in the brain by elevating their extracellular levels. Notably, it does not increase dopamine release in the striatal complex, including the nucleus accumbens. At nanomolar concentrations, Pitolisant functions as an inverse agonist at H3 receptors, enhancing the endogenous histamine release above baseline levels, which underlies its wake-promoting effects.

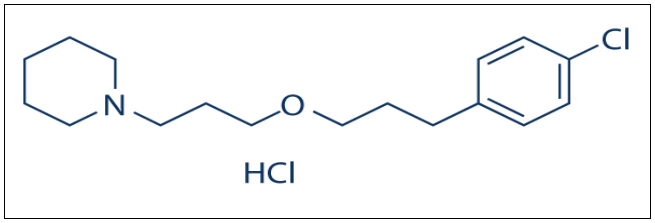

Fig 1: Chemical structure of Pitolisant HCl

A thorough literature survey revealed that no existing method has been reported for the estimation of Pitolisant in bulk and tablet dosage forms utilizing zero order and first order

derivative UV spectrophotometric techniques. Therefore, the primary objective of the present study was to develop novel, simple, precise, and economical UV spectrophotometric methods for the quantitative assay of Pitolisant in bulk and tablet formulations in accordance with International Council for Harmonisation (ICH) guidelines. The developed analytical methods were subsequently validated following the prescribed ICH validation protocols to ensure reliability and reproducibility for routine quality control application [6, 7].

2. Materials and Methods: Instrumentation: The analysis was performed using a Shimadzu UV-Visible double beam spectrophotometer (Model UV-1700), featuring a spectral bandwidth of 1 nm, which ensures high resolution and precise absorbance measurements. All weighing procedures were conducted using an electronic analytical balance (Model Shimadzu AUX-220) to ensure accurate sample preparation and measurement consistency.

Reagents and Chemicals

All reagents and chemicals utilized in this study were of HPLC grade and procured from Qualigens India Pvt. Ltd., Mumbai, India, ensuring high purity and analytical reliability. The pharmaceutical formulation investigated consisted of Pitolisant tablets, each containing an equivalent of 4.45 mg of Pitolisant, sourced from a reputable pharmaceutical manufacturer. Analytical method development focused on both zero order and first order derivative UV spectrophotometric techniques to achieve accurate quantification.

Preparation of Standard stock solution

An accurately weighed quantity of 44.5 mg of Pitolisant was transferred into a 10 mL volumetric flask, dissolved completely, and the volume was made up to the mark with distilled water to prepare a stock solution with a concentration of 4450 µg/mL.

Preparation of Working Standard Solution

A working standard solution of 445 µg/mL was prepared by accurately pipetting 1 mL of the stock solution into a 10 mL volumetric flask and diluting to the mark with distilled water to achieve the desired concentration.

Selection of solvent: Approximately 44.5 mg of Pitolisant was evaluated for its solubility in a range of solvents, including distilled water, ethanol, methanol, dichloromethane, acetonitrile, toluene, chloroform, and cyclohexane. Among these, Pitolisant exhibited the greatest solubility in distilled water, which was therefore selected as the solvent for subsequent analyses.

Selection of Analytical Wavelength for Measurement

From the working standard solution (445 µg/mL), 1 mL was further diluted to 10 mL with distilled water to prepare a 44.5 µg/mL solution. This solution was scanned over the wavelength range of 200-400 nm using distilled water as the blank. The maximum absorbance (λmax) for Pitolisant was observed at 221 nm, as illustrated in Figures 2 and 3.

Preparations of sample solutions

Twenty tablets were weighed to determine the average tablet weight, after which the tablets were finely powdered. An amount of the powder equivalent to 4.45 mg of Pitolisant was accurately transferred into a 10 mL volumetric flask containing 5 mL of distilled water, followed by sonication for 30 minutes to ensure complete dissolution. The flask was left at room temperature for 5 minutes, and the volume was then adjusted to the mark with distilled water to prepare a sample stock solution of 445 µg/mL. The solution was subsequently filtered through Whatman filter paper to remove any insoluble excipients. For further analysis, 1 mL of the filtered solution was diluted to 10 mL with distilled water.

3. Method Validation for Zero Order and First Order

Linearity and Calibration Curve

Linearity was assessed by plotting absorbance against concentration, with the correlation coefficient (r^2) derived from linear regression analysis, as illustrated in Figure 4. Aliquots of the Pitolisant working standard solution (445 µg/mL) at volumes of 0.5, 1.0, 1.5, 2.0, and 2.5 mL were accurately transferred into separate 10 mL volumetric flasks and diluted to volume with distilled water, yielding concentrations of 22.25, 44.5, 66.75, 89, and 111.25 µg/mL, respectively. Absorbances were recorded for zero order at 221 nm and first order derivative at 228nm against distilled water blank, and calibration plots are presented in Figures 4 and 5.

Precision

Precision studies were conducted as follows

- **Repeatability**: A 44.5 µg/mL solution was prepared by diluting 1 mL of the working standard to 10 mL with distilled water. Absorbance at 221 nm was measured six times, and relative standard deviation (%RSD) was calculated.
- **Intra-day Precision**: The 44.5 µg/mL solution was analyzed six times within the same day, and %RSD values were computed.
- **Inter-day Precision**: The same solution was analyzed six times across different days with corresponding %RSD values determined.

Accuracy: Accuracy was evaluated using the standard addition method by spiking known concentrations (22.25, 44.5, and 66.75 µg/mL) of Pitolisant into a pre-analysed sample solution (44.5 µg/mL). Triplicate analyses were performed for each mixture, and percentage recoveries were calculated from absorbance measurements.

Ruggedness: Ruggedness was tested by analyzing aliquots of a homogeneous sample by two different analysts under varying laboratory and environmental conditions to confirm method reproducibility.

Limit of Detection and Limit of Quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to ICH guidelines using the equations: The following equations were applied:

$$LOD = 3.3 \times \sigma / s$$
$$LOQ = 10 \times \sigma / s$$

Where,

σ = the standard deviation of response

s = the slope of the calibration curve

Forced degradation studies: Stability studies form an essential part of pharmaceutical analysis, offering insights into the integrity, potency, and safety of drug substances and formulations throughout their shelf life. As per ICH guidelines, forced degradation studies are conducted to explore the degradation pathways and intrinsic stability of drug molecules when exposed to different stress conditions such as acidic, alkaline, oxidative, reductive, and photolytic environments.

UV spectrophotometry serves as a simple, quick, and economical analytical method commonly employed for such evaluations. In this study, the active pharmaceutical ingredient (API) was examined using UV spectrophotometry at its maximum absorption wavelength (λmax) of 221 nm.

Acid degradation [7]

A 1 mL aliquot of the Pitolisant working standard solution (445 µg/mL) was transferred to a 10 mL volumetric flask, followed by the addition of 1 mL of 0.1 N sulfuric acid (H_2SO_4). The mixture was left undisturbed for 24 hours, then neutralized by adding 1 mL of 0.1 N potassium hydroxide (KOH). The solution was diluted to volume with distilled water, and absorbance was recorded at 221 nm.

Alkali degradation [7]

A 1 mL of the working solution was combined with 1 mL of 0.1 N KOH in a 10 mL volumetric flask. After 24 hours of incubation, the solution was neutralized with 1 mL of 0.1 N H_2SO_4, diluted to the mark with distilled water, and the absorbance measured at 221 nm.

Oxidative degradation [7]

To induce oxidative stress, 1 mL of the working solution was mixed with 1 mL of 0.3% hydrogen peroxide (H_2O_2) and kept for 24 hours. The final volume was adjusted with distilled water, and absorbance was recorded at 221 nm.

Reduction degradation [8]

A 1 mL portion of the working standard was treated with 1 mL of 10% sodium bisulfite solution to induce reduction degradation. The mixture was left for 24 hours, diluted to volume, and analysed at 221 nm.

Photolytic degradation [8]

For photostability evaluation, raw material was exposed to direct sunlight for 4 hours. Subsequently, a 445 µg/mL solution was prepared from the treated material. An aliquot of 1 mL was diluted to 10 mL with distilled water, and absorbance was measured at 221 nm.

4. Results and Discussion: The developed analytical method was validated in accordance with the International Council for Harmonisation (ICH) guidelines, which define the key parameters for analytical validation, including linearity, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ), and ruggedness. The UV spectra of Pitolisant corresponding to the zero-order and first-order derivative measurements are illustrated in Figures 2 and 3.

A series of five different concentrations of Pitolisant, ranging from 22.25 to 111.25 µg/ml, were prepared. Absorbance was measured at 221 nm for the zero-order and at 228 nm for the first-order derivative spectra. The respective calibration curves are depicted in Figures 4 and 5. To ensure reproducibility, calibration curve analysis was performed six times at the selected wavelengths. Graphs were plotted between absorbance and concentration, and optical parameters such as correlation coefficient, LOD, LOQ, and standard error were calculated.

The correlation coefficients were found to be 0.9997 for the zero-order method and 0.9998 for the first-order derivative, confirming excellent linearity for both methods. The summarized optical parameters are presented in Tables 1 and 6.

For assay studies, a marketed tablet formulation equivalent to 4.45 mg of Pitolisant was analysed. Based on linearity data, a 44.5 µg/ml solution was prepared, and its absorbance was measured at 221 nm. The drug content in six replicate test solutions was determined, showing percentage purity values of 99.50%±0.5197 for the zero-order and 99.73% ± 0.5576 for the first-order derivative methods. The %RSD values remained below 2%, indicating high precision and reliability (Tables 2 and 7).

The precision of the method was further assessed through intra-day and inter-day studies. For the zero-order method, %RSD values were 0.5865 and 0.5423, whereas for the first-order derivative they were 0.4110 and 0.4941, respectively demonstrating excellent repeatability and intermediate precision (Tables 3 and 8).

Accuracy was confirmed by recovery studies, where the percentage recovery of Pitolisant ranged from 99.02% to 100.67% for the zero-order method and from 99.73% to 101.43% for the first-order derivative method. The low %RSD values further confirmed that the developed UV spectrophotometric methods are accurate and reliable (Tables 4 and 9).

Ruggedness of the methods was evaluated under different conditions. For the zero-order method, %RSD values for Instrument 1 and Instrument 2 were 0.6394 and 0.5992, while for the first-order derivative method, they were 0.2415 and 0.2592. Similarly, %RSD values for Analyst 1 and Analyst 2 were 0.9479 and 0.1425 for the zero-order, and 0.2370 and 0.2075 for the first-order derivative method. The consistently low %RSD values across both instruments and analysts confirmed the ruggedness and reproducibility of the developed UV methods (Tables 5 and 10).

5. Figures and Tables

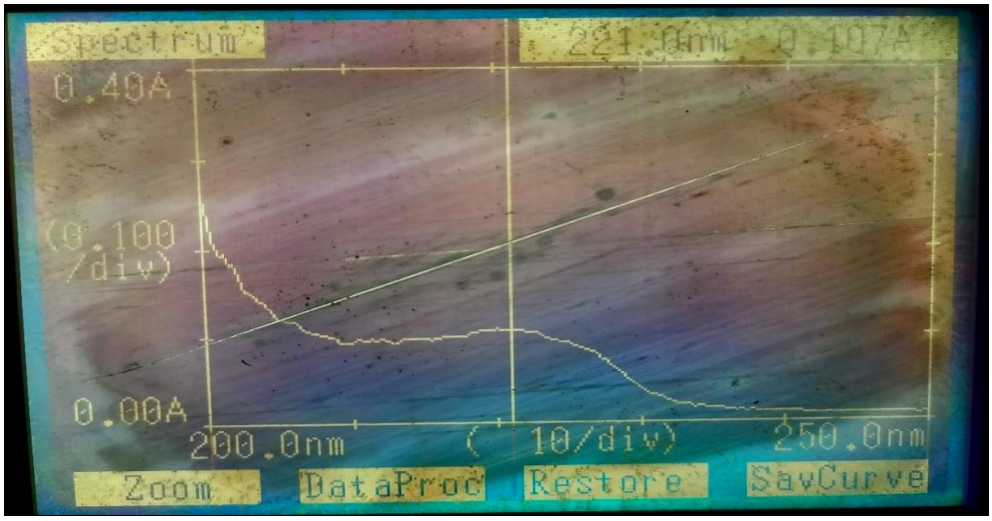

Fig 2: Zero order spectrum of Pitolisant

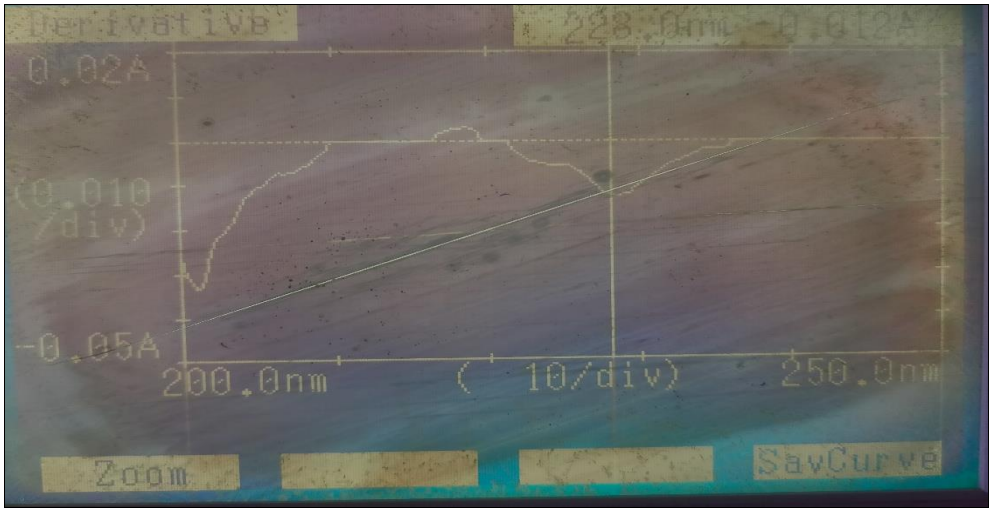

Fig 3: First order spectra of Pitolisant

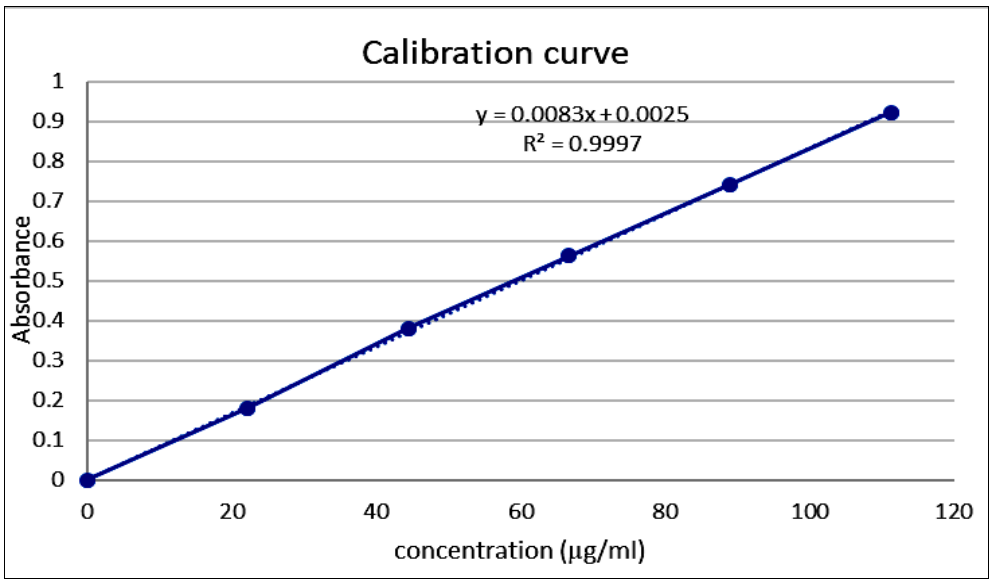

Fig 4: Calibration graph of Pitolisant for zero order

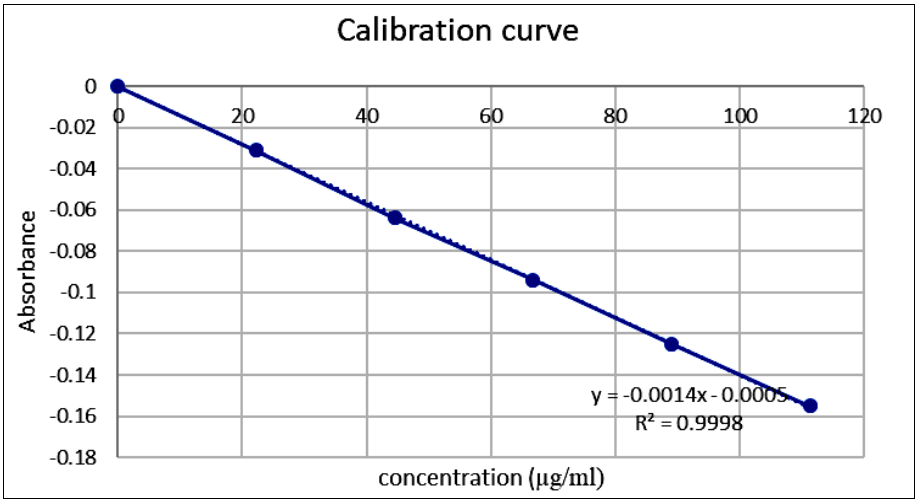

Fig 5: Calibration graph of Pitolisant for first order

Table 1: Optical Characterisation of Pitolisant (Zero Order)

Parameters	Zero order method - Pitolisant (221nm)
Beer's law range (µg/ml)	22.25 - 111.25
Regression equation (y=mx + c)	y = 0.0083x + 0.0025
Slope	0.0083
Intercept	0.0025
Correlation coefficient (r^2)	0.9997
Standard error	0.0046
Limit of detection (µg/ml)	0.7566
Limit of quantification (µg/ml)	2.2928

Table 2: Precision of Pitolisant Formulation (Zero Order Method)

Sample	Sample number	Label claim (mg/tab)	Amount present (mg/tab)	% purity	Mean purity	SD	% RSD
Pitolisant	1	4.45	4.45	100.00	99.95	0.5195	0.5197
	2	4.45	4.42	99.32			
	3	4.45	4.47	100.44			
	4	4.45	4.44	99.77			
	5	4.45	4.48	100.67			
	6	4.45	4.43	99.55			

Table 3: Intraday and Interday of Pitolisant Formulation (Zero Order Method)

Sample	Sample number	Label claim (mg/tab)	% purity		SD		%RSD	
			Intraday	Interday	Intraday	Interday	Intraday	Interday
PITO	1	4.45	100	99.70	0.5871	0.5434	0.5865	0.5423
	2	4.45	100.67	100				
	3	4.45	100.54	100.80				
	4	4.45	99.50	100.54				
	5	4.45	100.80	99.50				
	6	4.45	99.49	100.67				

PITO-Pitolisant*

Table 4: Recovery Study (Zero Order Method)

Sample	% Concentration	Sample amount (µg/ml)	Amount spiked (µg/ml)	Estimated amount (µg/ml)	Recovered amount (µg/ml)	Average % Recovery*	SD	%RSD
PITO	50	44.5	22.25	66.9	22.4	100.67	1.3863	1.3609
	100	44.5	44.5	88.8	44.34	99.55	0.2650	0.2660
	150	44.5	66.75	110.6	66.1	99.02	0.1755	0.1770

* Mean of 3 Observations

Table 5: Ruggedness Study of Pitolisant Formulation **(**Zero Order Method)

Sample	Type of ruggedness	Average % obtained*	SD	% RSD
Pitolisant	Analyst-1	99.81	0.9461	0.9479
	Analyst-2	99.58	0.1420	0.1425
	Instrument-1	101.10	0.6465	0.6394
	Instrument-2	99.90	0.5989	0.5992

* Mean of 6 Observations

Table 6: Optical Characterisation of Pitolisant (First Order Derivative)

Parameters	First order derivative method - Pitolisant (228 nm)
Beer's law range (µg/ml)	22.25-111.25
Regression equation (y=mx + c)	y = -0.0014x - 0.0005
Slope	-0.0014
Intercept	0.0006
Correlation coefficient (r^2)	0.9998
Standard error	0.0006
Limit of detection (µg/ml)	3.7813
Limit of quantification (µg/ml)	11.4601

Table 7: Precision of Pitolisant Formulation (First Order Derivative)

Sample	Sample number	Label claim (mg/tab)	Amount present (mg/tab)	% purity	Mean purity	SD	% RSD
Pitolisant	1	4.45	4.46	100.22	99.73	0.5561	0.5576
	2	4.45	4.41	99.10			
	3	4.45	4.43	99.55			
	4	4.45	4.41	99.10			
	5	4.45	4.46	100.22			
	6	4.45	4.46	100.22			

Table 8: Intraday and Interday of Pitolisant Formulation (First Order Derivative)

Sample	Sample number	Label claim (mg/tab)	% purity		SD		%RSD	
			Intraday	Interday	Intraday	Interday	Intraday	Interday
PIT	1	4.45	99.6	100.2	0.4098	0.4929	0.4110	0.4941
	2	4.45	100.2	99.3				
	3	4.45	99.3	99.3				
	4	4.45	100.2	100.2				
	5	4.45	99.6	99.3				
	6	4.45	99.3	100.2				

Table 9: Recovery Study (First Order Derivative)

Sample	% Concentration	Sample amount (µg/ml)	Amount spiked (µg/ml)	Estimated amount (µg/ml)	Recovered amount (µg/ml)	Average % Recovery*	SD	%RSD
PITO	50	44.5	22.25	66.7	22.2	99.77	1.2124	1.2160
	100	44.5	44.5	89.64	45.14	101.43	1.1124	1.0970
	150	44.5	66.75	111.07	66.57	99.73	0.1101	0.1103

* Mean of 3 Observations

Table 10: Ruggedness Study of Pitolisant Formulation (First Order Derivative)

Sample	Type of ruggedness	Average % obtained*	SD	% RSD
Pitolisant	Analyst-1	99.8	0.2366	0.2370
	Analyst-2	99.86	0.2073	0.2075
	Instrument-1	99.71	0.2408	0.2415
	Instrument-2	99.81	0.2488	0.2592

* Mean of 6 Observations

Forced degradation studies for the zero-order method

Pitolisant was exposed to forced degradation studies under acidic, alkaline, oxidative, reductive, and photolytic stress conditions, where the drug remained stable in all tested environments. These findings confirm that Pitolisant is stable under acidic, alkaline, oxidative, reductive, and photolytic stress. The developed zero-order UV spectrophotometric method effectively detected the stability, confirming its reliability for routine stability evaluation of bulk drug and tablet formulation.

Table 11: Pitolisant Forced degradation studies results in different parameters

Stress conditions	Time interval	% degradation
Acidic degradation	24 hrs	Not degraded
Alkali degradation	24 hrs	Not degraded
Oxidative degradation	24 hrs	Not degraded
Reduction degradation	24 hrs	Not degraded
Photolytic degradation	4 hrs	Not degraded

Advantages of the developed method

- Simple and easy to perform with minimal sample preparation.
- Cost-effective, using only distilled water as a solvent.
- Rapid analysis suitable for routine quality control.
- Accurate and precise, with % RSD values well below 2 %.
- Rugged and reproducible under varied conditions.

Conclusion

The developed zero-order and first-order derivative UV spectrophotometric methods are simple, rapid, precise, accurate, and economical for the quantification of Pitolisant in both bulk and tablet dosage forms. Both methods exhibited excellent linearity ($r^2 > 0.999$), high accuracy with recovery values close to 100%, and superior precision with %RSD values below 2%, confirming their reliability. The low LOD and LOQ values demonstrate adequate sensitivity, while ruggedness studies verified the consistency of results under varied analytical conditions. Forced degradation studies using the zero-order method revealed that Pitolisant remained stable under acidic, alkaline, oxidative, photolytic, and reductive stress conditions. Overall, the validated methods are robust, stability-indicating, and well-suited for routine quality control and stability evaluation of Pitolisant in pharmaceutical formulations.

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Authors Contributions

All the authors have contributed equally.

Conflict of Interests: Declared none.

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