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Research Article

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF HYDROXYZINE HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, rapid, precise, and economical UV spectrophotometric method was developed and validated for the estimation of Hydroxyzine Hydrochloride in marketed tablet formulation. The method was based on the measurement of absorbance at 230 nm using distilled water as solvent. Hydroxyzine Hydrochloride exhibited linearity in the concentration range of 5–40 µg/mL with a correlation coefficient (r^2) of 0.9986. The developed method was validated as per ICH Q2(R1) guidelines for parameters including linearity, accuracy, precision, specificity, robustness, limit of detection, and limit of quantification. The LOD and LOQ were found to be 0.179 µg/mL and 0.597 µg/mL, respectively. Accuracy studies showed satisfactory recovery of the drug, indicating the reliability of the method. Precision studies demonstrated %RSD values below 2%, confirming the precision of the method. Robustness studies revealed that minor deliberate variations in analytical conditions did not significantly affect the results, indicating method robustness. The proposed method was successfully applied for the assay of Hydroxyzine Hydrochloride in marketed tablets (Atarax® 10 mg), and the results were found to be within acceptable limits. The developed UV spectrophotometric method is simple, accurate, precise, robust, and suitable for routine quality control analysis of Hydroxyzine Hydrochloride in pharmaceutical formulations.

Keywords: UV spectrophotometry, Hydroxyzine Hydrochloride, Method development, Method validation, ICH Q2(R1).

INTRODUCTION

Hydroxyzine hydrochloride (HZ HCl) is chemically known as 2-[2-[4-[(4-chlorophenyl)-phenylmethyl] piperazin-1-yl] ethoxy] ethanol; dihydrochloride (Figure 1). Hydroxyzine is a first-generation histamine H1-receptor antagonist of the diphenylmethane and piperazine classes that exhibits sedative, anxiolytic, and antiemetic properties. (Tripathi, 2018, Indian Pharmacopoeia Commission, 2022, United States Pharmacopeia, 2011, United States Pharmacopeia, 2020). The drug acts on the central nervous system, on muscles, on exocrine glands and on capillary permeability (Altamura *et al*., 2013, Altamura *et al*., 2013). When administered via the traditional route, HZ HCL exhibits therapeutic effects within 15 to 30 minutes, being swiftly absorbed from the gastrointestinal tract. After 2 hours, peak therapeutic effects are observed, with an average duration of action lasting between 3 and 4 hours. (Brunton *et al*., 2018, Parisi *et al*., 2020)] HZ HCl is prescribed frequently to treat anxiety, tension, nausea, allergy, andto enhance the actions of opioid analgesics. As an H1 receptor antagonist, hydroxyzine blocks histamine at the peripheral H1 receptors, thus causing decreased allergic symptoms like rashes and itching. (Wishart *et al*., 2025) Owing to its broad therapeutic application, the accurate quantification of Hydroxyzine Hydrochloride in pharmaceutical dosage forms is essential to ensure quality, safety, and efficacy. UV-Visible spectrophotometry remains one of the most widely employed analytical techniques in pharmaceutical analysis due to its simplicity, rapidity, cost-effectiveness, and minimal solvent consumption. It is particularly suitable for routine analysis in quality control laboratories, especially where resources

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are limited (Beckett & Stenlake, 2002, Skoog *et al*., 2007, Chauhan *et al*., 2015). Safila Naveed and Fatima Qamar (2015) reported a simple UV spectrophotometric assay method for the estimation of Hydroxyzine Hydrochloride (Naveed & Qamar, 2015) However, the reported study primarily focused on linearity parameters, and comprehensive method validation parameters such as accuracy, precision, sensitivity (LOD and LOQ), and robustness as per International Council for Harmonisation (ICH) guidelines were not included.

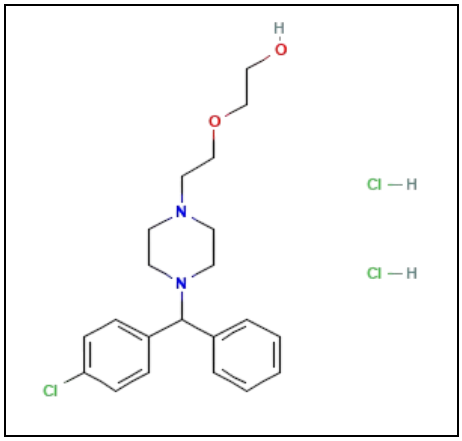

Figure 1. Chemical structure of Hydroxyzine Hydrochloride**.**

According to ICH Q2(R1) guidelines, validation of analytical methods is essential to establish the reliability, reproducibility, and suitability of the method for its intended purpose. (International Council for Harmonisation, 2022, International Council for Harmonisation, 2005). Therefore, there exists a need to develop and validate a simple, sensitive, accurate, and precise UV spectrophotometric method for the estimation of Hydroxyzine Hydrochloride with complete validation parameters. The present study aims to address this gap by developing and validating a UV spectrophotometric method for Hydroxyzine Hydrochloride in bulk drug and marketed tablet formulation in accordance with ICH Q2(R1) guidelines, making it suitable for routine quality control analysis.

MATERIALS AND METHODS

Materials

Hydroxyzine Hydrochloride API was obtained as a gift sample from Emcure Pharmaceutical Company (Purity > 98.2%). Marketed tablet formulation containing Hydroxyzine Hydrochloride (Atarax® tablets, labelled claim 10 mg) was procured from the local pharmacy. Distilled water was used as solvent throughout the analysis.

Instrumentation

UV spectrophotometric analysis was performed using a Shimadzu UV–Visible spectrophotometer (Model UV-1800) equipped with UV Probe software and matched 1 cm quartz cells.

Method development

Selection of solvent

Based on the solubility profile of HZ HCl, distilled water was selected as the solvent for UV analysis. The drug is freely soluble in water, and the solvent was transparent in the UV range, ensuring no interference at the analytical wavelength (λmax) of the drug and thereby getting reliable absorbance measurements.

Preparation of stock and working standard solutions

10mg of HZ HCl API was weighed carefully and dissolved in 5ml distilled water in a volumetric flask and diluted to 10ml mark on the volumetric flask with the same solvent to produce a 1000µg/ml stock solution. 100µg/ml solution was produced by withdrawing 1ml of stock solution and diluting it to 10ml with distilled water. Subsequent dilutions were made from this 100µg/ml solution to achieve 5, 10, 15, 20, 25, 30, 40µg/ml and volume make up was done by distilled water.

Determination of wavelength

Using distilled water as blank, a solution of 10µg/ml of HZ HCl was scanned between 200-400nm. The wavelength at which the drug showed highest absorbance was recorded. 230nm was fixed as the determination wavelength for HZ HCl.

Analysis of marketed formulation

A 10ml volumetric flask was filled with a precisely weighed quantity of Tablet powder (68mg) that equated to 10mg of HZ HCl. Then 7ml of distilled water was added to the flask and then it was sonicated for 15 minutes. Then

volume make up till the mark was done by adding distilled water. Whatman filter paper was used to filter this mixture. A 100µg/ml stock solution was obtained by diluting 1ml of the above filtrate with distilled water in a 10ml volumetric flask. 1ml was withdrawn from above stock solution and diluted to 10ml with the same solvent to produce 10µg/ml solution. The final dilution was then examined using recently developed UV-Visible Spectroscopy method and the drug content was reported as percentage.

Method Validation

The UV method was Validated as per the ICH guidelines Q2(R1).

Linearity and Range

Three replicates of the dilutions with a linear relationship between absorbance and concentration were assessed at the determined wavelength, covering the concentration range between 5-40µg/ml of HZ HCl. Calibration curve was plotted, regression equation and r2 value was determined from the above data.

Limit of Detection and Quantification

The obtained regression equation was used for the calculation of LOD and LOQ.

$LOD=3.3*\sigma/s$

$LOQ=10*\sigma/s$

Where, σ = Standard deviation

S = Slope of the corresponding calibration plot.

Accuracy

Synthetic mixtures of the marketed formulation containing known amounts of HZ HCl at different levels (80%, 100%, and 120%) were prepared for recovery experiments. The developed method was used to analyze the prepared samples, with each sample being measured in triplicate. The results included both the percentage recovery and the amount of drug present.

Precision

A 10 µg/ml dilution of HZ HCl was examined in triplicate over three days to achieve intermediate precision and six repetitions on the same day for repeatability and the outcome was presented as %RSD, SD, and % Drug Content.

Robustness

The robustness analysis was performed to evaluate the impact of a minor but deliberate changes in the spectrometric condition of HZ HCl in the dosage form of tablets. The wavelength at which the final diluted solution containing 10µg/ml of HZ HCl measured was ±2 nm from their λmax.

Specificity

The aim of specificity is to achieve a result that is detailed enough to allow an accurate evaluation of the analyte's strength or concentration in a sample. The overlay technique was used to evaluate the specificity of the prepared standard mixture and the final diluted solution containing 10µg/ml of HZ HCl.

Stability

The prepared standard as well as sample solutions which contain 10µg/ml of HZ HCl were checked for stability.

RESULTS AND DISCUSSION

The tablet sample solution was subjected to analysis by using the developed UV Spectroscopy method. The absorbance of sample solution of concentration 10µg/ml was recorded at λmax 230nm. The mean drug content determined from three replicate readings was 91.14 ± 1.05%), with a relative standard deviation (RSD) of 1.15%. It was found to be within the IP Limits 90-110%. (Table 1).

Table 1. Assay of HZ HCl in Marketed tablet formulation.

Brand name	Label claim (mg/tablet)	Amount found (mg)	%Assay ± SD
Atarax®	10	9.11	91.1±1.04

* Average of 3 estimations (n=3)

Linearity and Range

The linearity of the UV spectrophotometric method that was developed was assessed across a concentration range of 5-40 µg/ml. A calibration curve was generated by graphing absorbance against concentration. The method demonstrated outstanding linearity, achieving a correlation coefficient (r^2) of 0.9986. The linear regression equation was determined to be: y=0.0335x+0.03. The linearity results are presented in Table 2, and the calibration curve is shown in Figure 2. (UV spectra overlay in Figure 3).

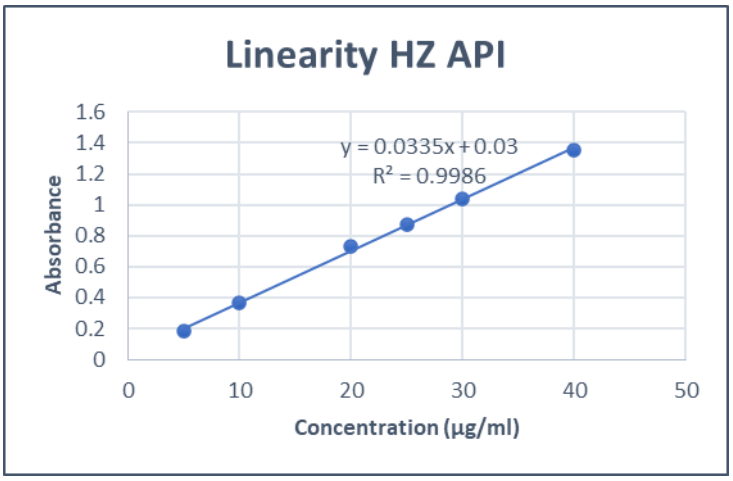

Figure 2. Calibration curve of Hydroxyzine hydrochloride

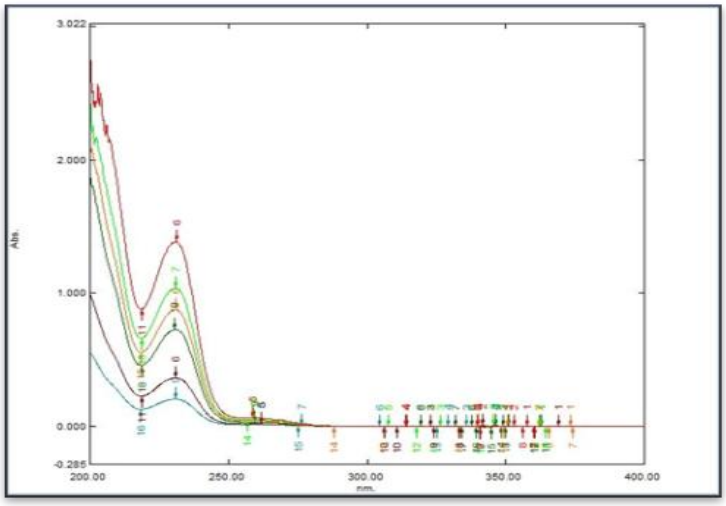

Figure 3. Linearity UV Spectra of HZ HCl.

Table 2. Linearity data for HZ HCl by UV Spectrophotometric method.

Concentration (µg/ml)	Mean absorbance ± SD
5	0.182 ± 0.002
10	0.363 ± 0.004
20	0.728 ± 0.004
25	0.87 ± 0.012
30	1.041 ± 0.006
40	1.355 ± 0.023

*Average of 3 estimations (n=3)

Limit of Detection and Quantification

The LOD and LOQ were found to be 0.179 µg/mL and 0.597 µg/mL, respectively, indicating good sensitivity of the method.

Accuracy

The accuracy of the developed UV spectrophotometric method was assessed by recovery studies at 80%, 100%, and 120% levels. The percentage recovery of Hydroxyzine Hydrochloride was found in between 93-95% w/w and within acceptable limits with %RSD less than 2, confirming the accuracy of the method (Table 3).

Table 3. Recovery study of HZ HCl.

% Recovery level	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery ± SD
80	8	16.925	94.02 ± 0.58
100	10	18.95	94.77 ± 1.66
120	12	20.83	94.70 ± 0.95

*Average of 3 estimations (n=3)

Precision

The precision of the developed UV spectrophotometric method was evaluated in terms of repeatability and intermediate precision. Repeatability was evaluated by analyzing six replicates of a 10 µg/mL drug solution under the same experimental conditions, yielding a %RSD of 1.24, which indicates excellent repeatability (Table 4).

Table 4. Repeatability (Intra-day precision) of HZ HCl by UV method.

Component	Amount taken (µg/ml)	Amount found (µg/ml) ± SD	%RSD
Hydroxyzine HCl	10	9.18 ± 0.11	1.24

*Average of 6 estimations (n=6)

Inter-day precision was assessed by analyzing a 10 µg/mL solution over three consecutive days in triplicate, with % RSD values of 0.68, 1.13, and 1.89, demonstrating acceptable reproducibility of the method (Table 5).

Table 5. Inter-day Precision of HZ HCl by UV method.

Day	Mean Drug content (µg/ml) ± SD	%RSD
1	9.06 ± 0.06	0.68
2	9.20 ± 0.10	1.13
3	9.63 ± 0.18	1.89

*Concentration=10(µg/ml); Average of 3 estimations (n=3)

Robustness

Robustness was evaluated by varying the detection wavelength by ±2 nm (228, 230, and 232 nm). The %RSD values at these wavelengths were 0.81, 0.32, and 0.93, respectively, indicating that minor wavelength variations do not significantly affect the method performance. (Table 6).

Table 6. Robustness study of HZ HCl by UV method.

Parameter varied	Condition	Mean Drug content (µg/ml) ± SD	%RSD
Wavelength (nm)	228	9.20 ± 0.07	0.81
	230	9.22 ± 0.02	0.32
	232	9.58 ± 0.08	0.93

*Concentration=10(µg/ml); Average of 3 estimations (n=3)

Specificity

Specificity was assessed by overlaying the UV spectra of the drug API solution and the tablet assay solution. Both spectra exhibited identical absorbance profiles with matching λmax at 230 nm, confirming that no spectral interference was present from excipients or formulation components. (Figure 4).

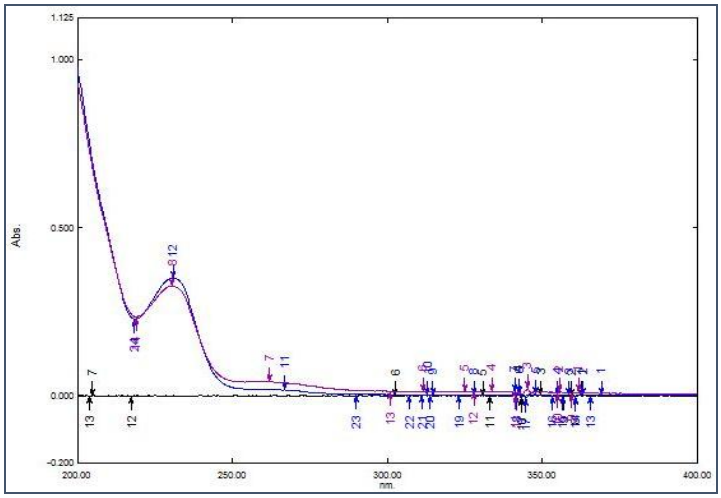

Figure 4. Overlay UV spectra demonstrating specificity of HZ HCl.

Stability

The prepared Standard HZ HCl solution was found to be stable for 24 hours. (Figure 5) (Table 7).

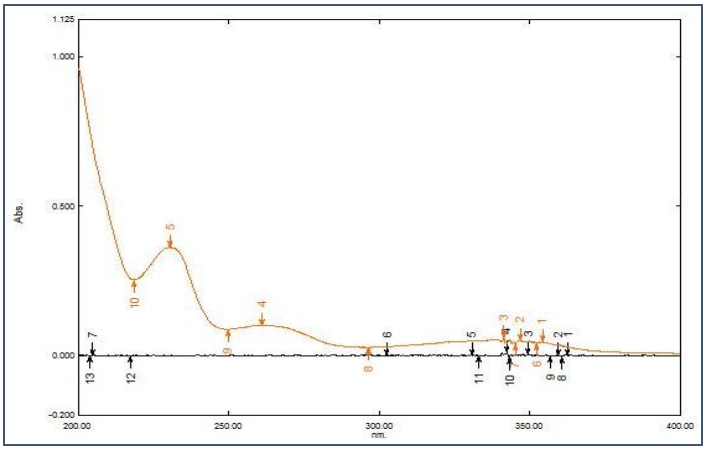

Figure 5. Stability UV spectra of HZ HCl at 24 hrs.

Table 7. Stability study of Hydroxyzine Hydrochloride standard solution at different time intervals.

Sr. No.	Time (Hrs)	Absorbance (nm)
1	0	0.361
2	6	0.339
3	12	0.326
4	18	0.316
5	24	0.314

CONCLUSION

A simple, rapid, precise and economical UV spectrophotometric method was developed and validated for the estimation of Hydroxyzine Hydrochloride in bulk and marketed tablet formulation. The method showed good linearity and was validated in accordance with ICH Q2(R1) guidelines for accuracy, precision, robustness, LOD, and LOQ. The method was successfully applied to the assay of Atarax® 10mg tablets, with results within acceptable limits, indicating its suitability for routine quality control analysis.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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