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ECO-FRIENDLY UV SPECTROPHOTOMETRIC METHOD FOR THE QUANTIFICATION OF HYDROXYZINE IN BULK DRUG AND PHARMACEUTICAL FORMULATION BY MULTIVARIATE CALIBRATION TECHNIQUE

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ABSTRACT

This research aims to establish an UV-Visible spectroscopic technique for Hydroxyzine by applying a multivariate regression equation that is precise, sensible, and reproducible method. Proposed technique depends on the equation of the linear regression performed by taking absorbance at five distinct wavelengths. Hydroxyzine maximum absorbance was obtained at 230nm using distilled water as the solvent. Graph obtained from concentration 7-13µg mL^{-1} resulted in linear curve and the regression coefficient was obtained as 0.9999. % RSD values for Intra-day, as well as Inter-day precision, was obtained as 1.057624 and 1.361254. The assay value determined was between 99.78% - 99.90% w/w.

Keywords: Hydroxyzine, sedative agent, UV spectrophotometry, Multivariate calibration, Assay, ICH guidelines

INTRODUCTION

Hydroxyzine hydrochloride (HDX) is chemically, (RS)-2-(2{4- [(4-chlorophenyl) HDX) **[1]**. Its molecular Formula is $C_{21}H_{29}C_{13}N_2O_2$ **[2]**. Its molecular weight is 447.83 g mol^{-1} **[3]**. It is an H1 receptor antagonist that belongs to the piperazine class and is used to treat secondary allergy diseases like Hepatocellular pruritus, atopic dermatoses, chronic urticaria, and contact dermatoses are examples of it. Moreover, it is employed for both pre- and post-anesthesia calming effects. Because of its antagonistic actions on several brain receptor systems, it possesses potent anxiolytic, mild anti-obsessive, and antipsychotic qualities **[4]**. A first-generation H1 receptor antagonist called HDX is still often given for generalized anxiety disorder **[5]**. A review of the literature suggests that hydroxyzine can be detected in biological fluids using a variety of techniques, including distinct gas chromatographic (GC) approaches, after being transformed into a benzophenone **[6]**. An electron-capture GC mass analysis is performed on this compound. Hydroxyzine hydrochloride has also been estimated using a high-performance liquid chromatographic (HPLC) technique with UV detection **[7]**. To calculate HDX, analytical techniques with differing degrees of difficulty and expense have been used. Typical examples consist of thin-layer chromatography **[8]**, high-performance liquid chromatography **[9]**, gas chromatography **[10]**, capillary zone electrophoresis **[11]**, acid–base titration **[12]**, conductometric titration **[13]**, differential pulse anodic voltammetry **[14]** and spectrophotometry **[15]**. It is official in pharmacopoeias like Indian Pharmacopoeia, British Pharmacopoeia and United States Pharmacopoeia **(Figure 1)**.

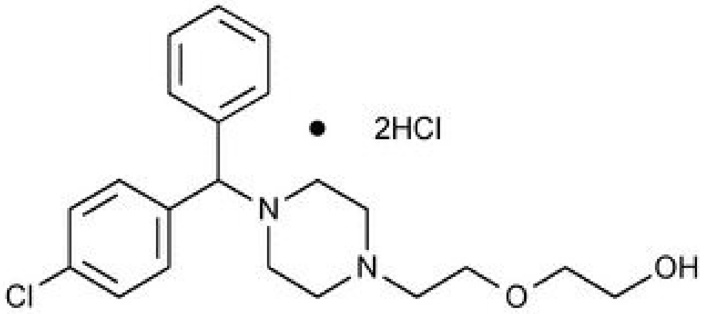

Figure 1: The chemical composition of Hydroxyzine

The suggested method directly assesses Hydroxyzine and has been verified with higher accuracy and precision than a traditional UV-Visible assay, results can be trusted more. This method can be utilized for bulk pharmaceuticals and many dosage forms and is more direct, quick, and affordable than previous approaches. A multivariate standardization procedure produced a particular outcome, and the conversion of the outcome yielded a dependent variable, "m." This technique offers excellent sensitivity, resolving power, expediency, and economic analytical efficiency for a determined quantification of HDX. HDX (X) refers to the absorbance of an analyte. It requires scanning seven distinct concentrations (λ = 224, 227, 230, 233 and 236 nm); for any desired wavelength, the following formula can then be used.

$A_{\lambda 224} = a\ X\ C_x + \ k_1$ -------------------- (1)

$A_{\lambda 227} = b\ X\ C_x + \ k_2$ --------------------- (2)

$A_{\lambda 230} = c\ X\ C_x + \ k_3$---------------------- (3)

$A_{\lambda 233} = d\ X\ C_x + \ k_4$-------------------- (4)

$A_{\lambda 236} = e\ X\ C_x + \ k_5$-------------------- (5)

Whereas absorbance of the analyte is denoted as A $_{\lambda}$, the analyte's slope of the linear regression functions are a, b, c, d, and e; The analyte's concentration is indicated as Cx, and the k1, k2, k3, k4, and k5 indicates the intercepts at the specific wavelengths.

The selected five wavelengths equation (1-5) listed above summarised in the following

$$A_T = a\ X\ C_x + \ b\ X\ C_x + \ c\ X\ C_x + d\ X\ C_x + \ e\ X\ C_x + \ K_T \text{-----------} (6)$$

The aforementioned equation can be further simplified to

$A_T = C_x\ (a + b + c + d + e) + \ K_T$---- (7)

The sums of the intercepts from regression equations at a chosen five wavelengths are denoted by A_T and K_T, respectively. The concentration of the analyte X is calculated using the formula given below **[16-22]**.

$$C_x = \frac{A_T - K_T}{(a+b+c+d+e)} \text{ -------------} (8)$$

Greenness Evaluation Techniques

The Globally Harmonized System of Classification and Labelling of Chemicals (GHS) established a set of pictograms with related signal words, and the analytical eco scale **[23]** is predicated on assigning penalty points relying over both quantity and number. The analytical eco scale approach considers each reagent, including its kind and quantity, potential occupational exposure, energy depletion, and waste. Penalty points are eliminated from a 100 point base score. Analytical eco-scale = 100 - total penalty points……………. (9)

The Green Analytical Procedure Index (GAPI) is a visual depiction made up of five pentagons with distinctive colour coding. The colour coding in the pictogram corresponds to three levels of evaluation at each stage of an analytical technique. The colour coding used by GAPI to determine greenness spans from green to yellow to red, denoting the low, medium, and high environmental impacts connected with the analytical technique, respectively. J. Potka Wasylka provided a succinct overview of GAPI in the year 2018 **[24]**. The third assessment methodology makes use of AGREE metrics' **[25]** special software for assessing the greenness profile. The result of the software is a circle with numbers around the edges that range from 1 to 12 and are oriented clockwise. These figures represent the 12 green analytical chemistry philosophies. Based on the inputs and their weight, the outputs of each of these 12 principles are rated from 0 to 1. This aggregate scale uses the colours red, yellow, and green to show different numbers. Red means zero, dark green means one or close to one, and yellow means a number between red and dark green. A score that represents the level of greenness is produced by adding the 12 principles and the core.

MATERIALS AND METHODS

Chemicals and reagents

- Distilled water
- Hydroxyzine API was ex-gratis from Ideal Analytical Laboratory, Puducherry
- The marketed tablet formulation used was Atarax manufactured by, Dr Reddy's Laboratories Ltd (Labelled to have 25 mg Hydroxyzine hydrochloride) purchased from a nearby market.

Instrumentation

- Double beam UV spectrophotometer (LAB INDIA 3092)
- The Ultra Sonicator
- Micro balance
- The Micropipette

Analytical method development

Solvent selection:

Hydroxyzine has been found to be readily soluble in distilled water. Therefore, both the standard and the sample were further diluted using distilled water.

Standard stock solution

A 25 mL volumetric flask containing 25 mg of the standard medication is diluted with distilled water to create the stock solution of hydroxyzine. Aliquots of this solution with concentrations, (7 – 13 µg mL^{-1}) were prepared and utilized for further analysis.

Determination of λ_{max}

Hydroxyzine's maximum absorbance is established using a solution made by dissolving the standard stock solution in water to a concentration of 10 µg mL^{-1}. The prepared solution was scanned in the UV-visible region between 200 and 400 nm. Obtained graph plot between the concentrations against absorbance gives a linear curve**.** The outcomes are examined around the spectrum range 230 nm, i.e., 224, 227, 230, 233, and 236 nm, for improving correlation and diminishing the oscillations of the instrument. **(Table 1, Table 2)**.

Sample solution preparation

Preparation of sample solution is done by taking twenty tablets of Hydroxyzine, precisely weighed and powdered. A 25 mL standard flask was filled with the weight equivalent to 25 mg, sonicated for 15 minutes, the sample was dissolved, and distilled water was added to make up the volume. This solution was then filtered, and used for further analysis.

Method Validation

The ICH guidelines have been followed in the validation of this method's sensitivity, precision, accuracy, and linearity **[26]**.

Linearity

Various concentrations ranging from 7-13 µg mL^{-1} were prepared from standard stock solutions. To minimize instrumental variations and enhance the correlation, these solutions were evaluated over a variety of wavelengths: 224, 227, 230, 233, and 236 nm **(Figure 3, Table 1)**.

Table 1: UV Calibration data at five distinct wavelengths

Concentration (µg mL^{-1})	Absorbance*				
	224 nm	227 nm	230 nm	233 nm	236 nm
7	0.174	0.199	0.210	0.194	0.166
8	0.209	0.235	0.250	0.235	0.184
9	0.235	0.273	0.290	0.268	0.214
10	0.278	0.316	0.330	0.309	0.255
11	0.301	0.350	0.370	0.342	0.283
12	0.337	0.388	0.410	0.380	0.311
13	0.370	0.424	0.449	0.416	0.337

#Average of 5 determinations; UV= Ultra violet

The graph is plotted as concentration against absorbance and standardizations were achieved. The sensitivity of the technique was established by calculating the limit of detection and quantification using the following formula.

LOD = 3.3 σ/S ………………….. (8)

LOQ = 10 σ /S.............................. (9)

Hereby, lowermost concentration of standard deviation (SD) and the standard curve of the slope is denoted as S.

Precision

In order to determine the intra-day and inter-day precision, a 10 µg mL^{-1} solution was made and scanned six times. Six different days were used to measure the intra-day precision and within the same day to measure the inter-day precision.

Accuracy

The recovery study for recommended technique were determined at 80%, 100%, and 120% by the standard addition technique, and using this % recovery was calculated. The solutions for the recovery study were prepared from both standard and sample stock solutions.

Assay

By measuring the absorbance at 230 nm from the extracted tablet solution, the amount of hydroxyzine present in the tablet was determined.

RESULTS AND DISCUSSION

As seen in **Figure 2**, the Hydroxyzine maximum absorbance was measured at 230 nm using water as the solvent.

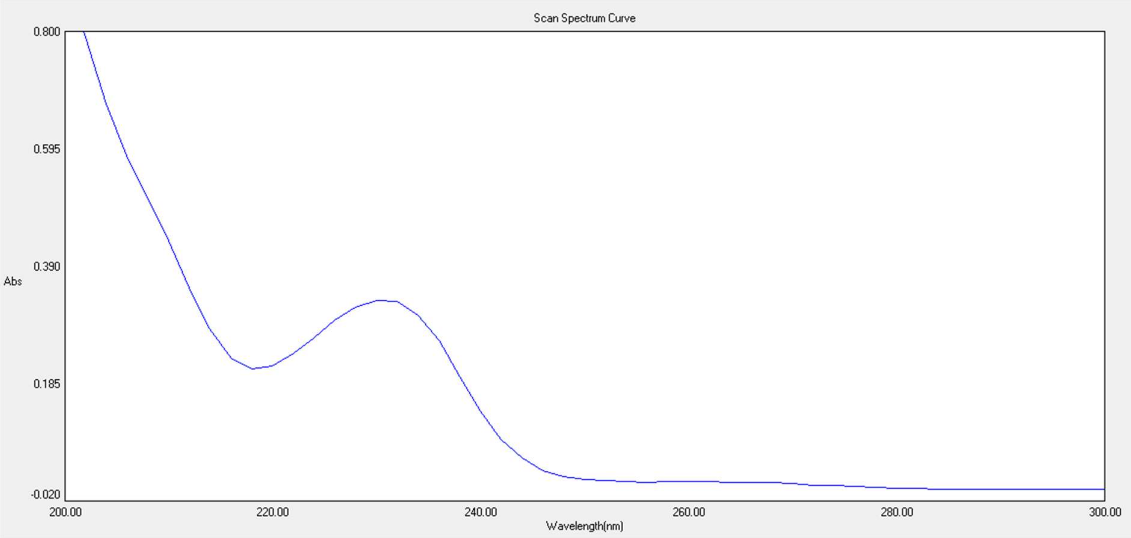

Figure 2: UV spectrum of Hydroxyzine (10 µg mL^{-1}), λ_{max} at 230 nm

Within the concentration range between 7 - 13 µg mL^{-1} this technique was found to be linear. An excellent linear correlation is obtained from the calibration plots with R^2 - 0.9993- 0.9999. The % relative standard deviation for precision obtained as 0.2487 and 0.6316. The detection and quantification limits were at 0.1627 and 0.4932 µg mL^{-1}, respectively. Hence the values come under validation parameters accordance to the ICH guidelines limitations.

Linearity

The linearity spectra is depicted in **Figure 3**and the corresponding calibration curves are shown from **Figure 4 to 8**. The approach is deemed accurate and dependable for every wavelength, as indicated by the low values of the relative standard deviation. The calculation of detection and quantification of limit has been done and results were depicted in **Table 2**.

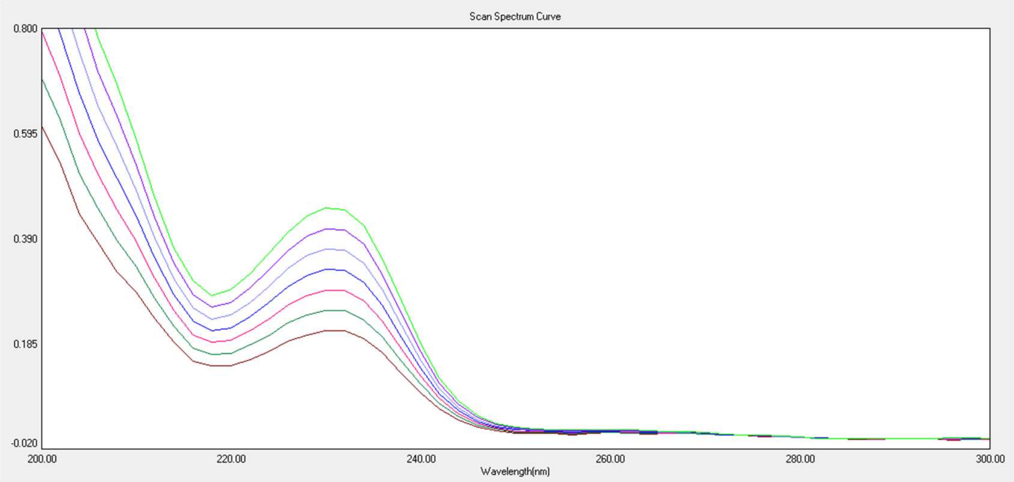

Figure 3: UV Spectrum of Hydroxyzine showing linearity at 230 nm

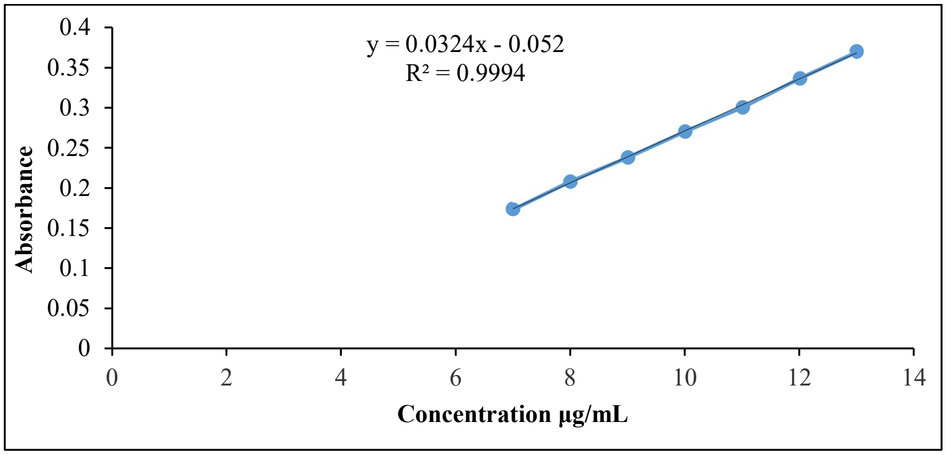

Figure 4: Calibration curve at 224 nm

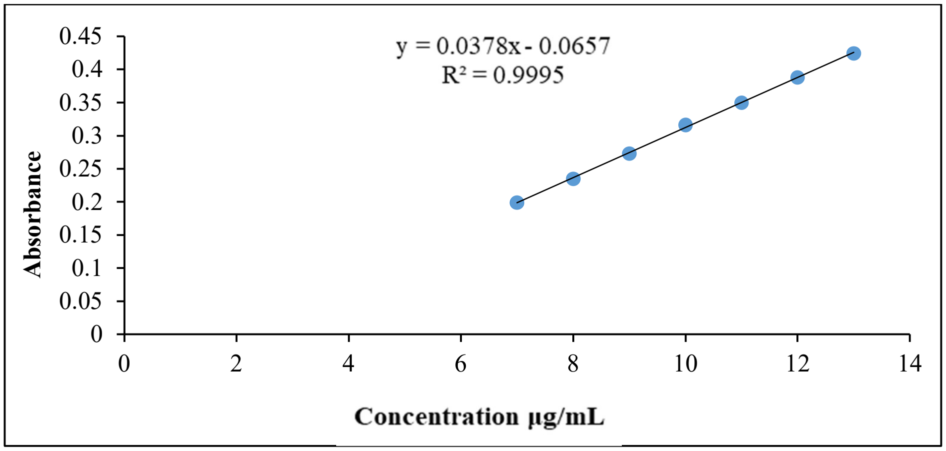

Figure 5: Calibration curve at 227 nm

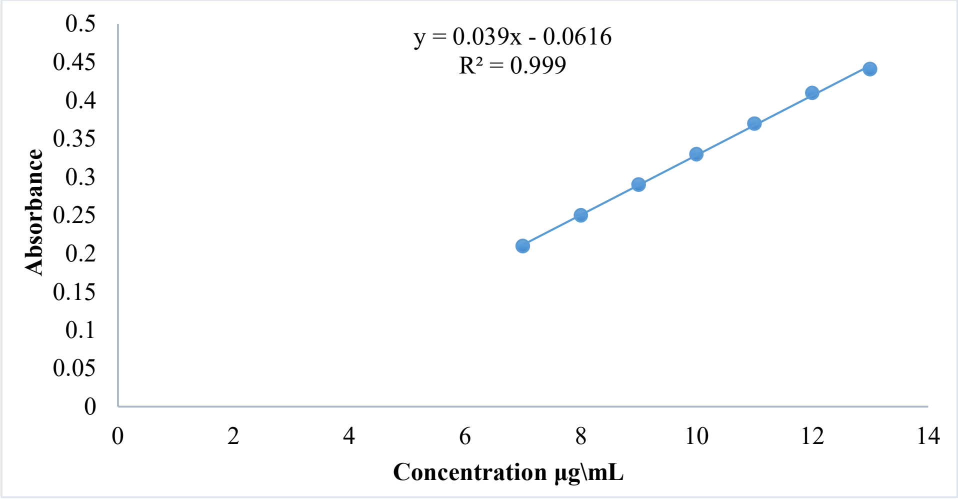

Figure 6: Calibration curve at 230 nm

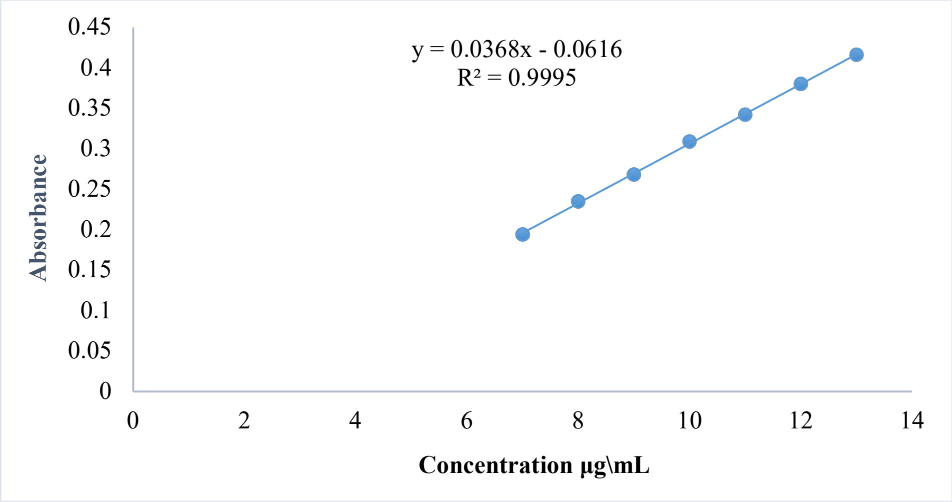

Figure 7: Calibration curve at 233 nm

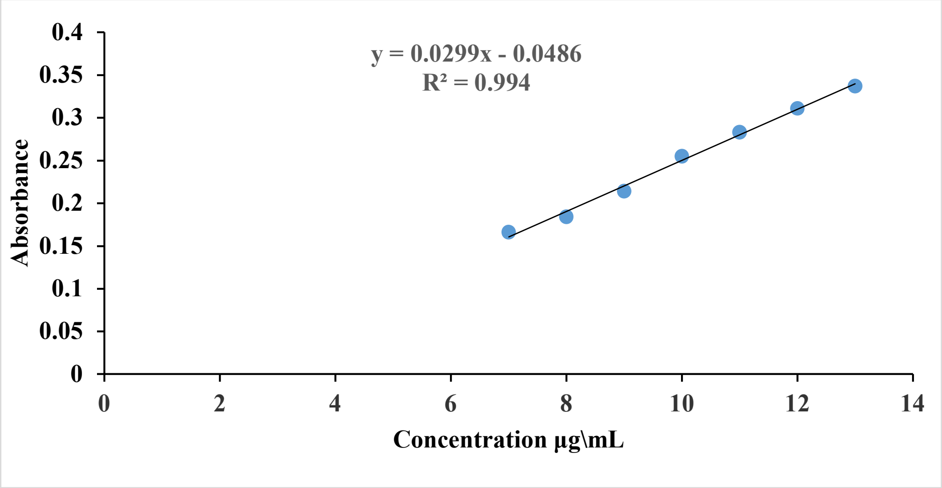

Figure 8: Calibration curve at 236 nm

Table 2: Linearity data with LOD and LOQ at selected five wavelengths

Wavelength (nm)	Regression equation	R^2	LOD (µg mL^{-1})	LOQ (µg mL^{-1})	% RSD
224	y = 0.0324x - 0.052	0.9994	0.1832	0.5553	0.6616
227	y = 0.0378x - 0.0657	0.9995	0.1814	0.5497	0.6654
230	y = 0.039x - 0.0616	0.9999	0.2490	0.7546	0.8962
233	y = 0.0368x - 0.0616	0.9995	0.1800	0.5456	0.6553
236	y = 0.0299x - 0.0486	0.9994	0.6082	1.8431	2.2012

***nm = nanometre; µg mL-^{1}= Microgram per millilitre**

Precision

The suggested technique is distinct, dependable, and accurate, as evidenced from the low standard deviation values. The intra-day precision and inter-day precision values are 0.9657 and 0.3493, respectively. It is within tolerances of less than 2% at every wavelength (**Figure 9, 10**).

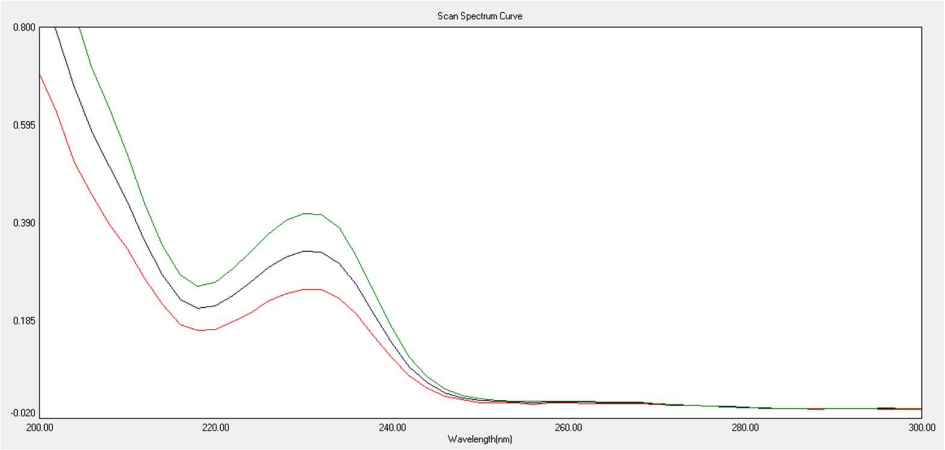

Figure 9: UV spectra showing intraday precision

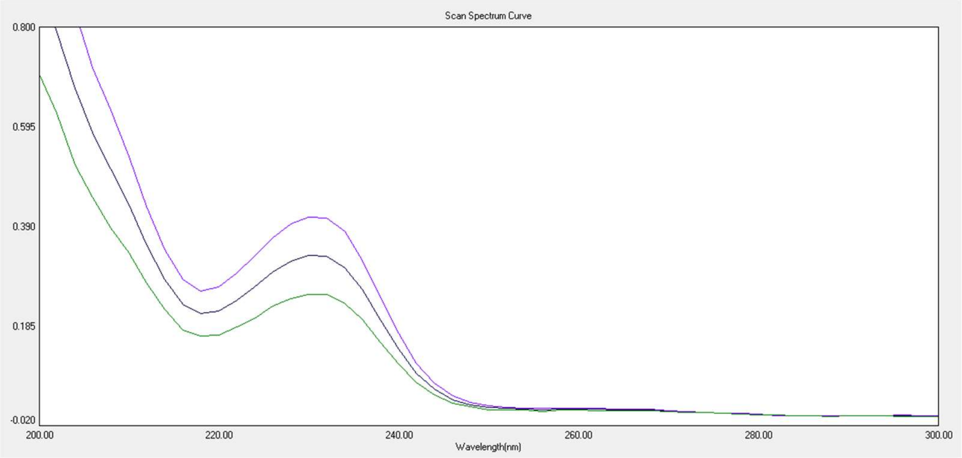

Figure 10: UV spectra showing interday precision

Recovery

The Hydroxyzine % recovery was found between 97% to 103% w/w, according to the ICH guidelines. The acceptable range of % recovery was from 97-103% w/w **(Figure 11, Table 3)**.

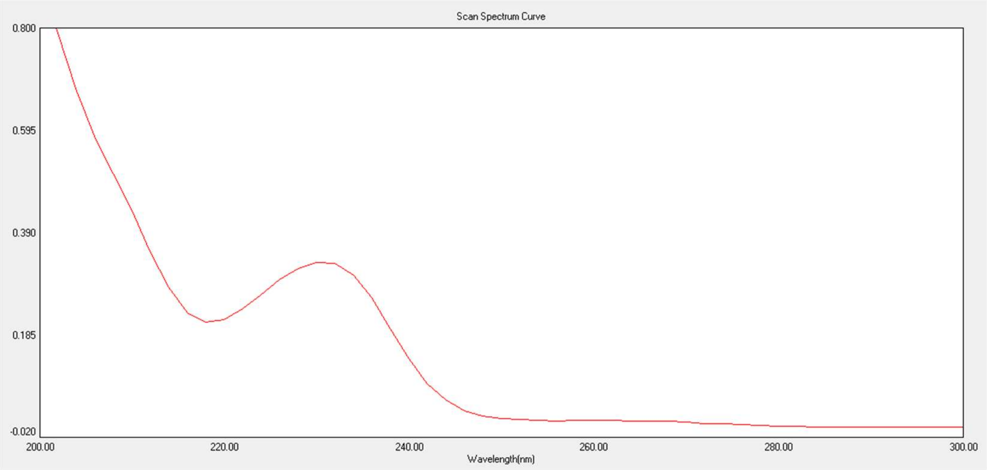

Figure 11: UV Spectrum showing accuracy of Hydroxyzine.

Table 3: Recovery Studies

Wavelength (nm)	Amount present (µg mL^{-1})	Amount added (µg mL^{-1})	Absorbance	Amount recovered (µg mL^{-1})	% Recovery
224 nm	5	3	0.21	8.06	100.75
		5	0.279	10.12	101.20
		7	0.337	11.98	99.85
227 nm	5	3	0.235	8.11	101.38
		5	0.316	9.98	99.80
		7	0.387	12.05	100.42
230 nm	5	3	0.253	7.86	98.25
		5	0.331	10.09	100.90
		7	0.411	12.24	102.00
233 nm	5	3	0.234	7.95	99.38
		5	0.309	10.19	101.90
		7	0.381	12.08	100.67
236 nm	5	3	0.183	8.05	100.63
		5	0.254	9.94	99.40
		7	0.315	11.89	99.08

Assay:

The Hydroxyzine maximum absorbance was measured at 230 nm for the tablet formulation by UV-Visible spectroscopy. The amount and assay percentages were obtained as 24.99 mg and 99.95 % w/w, further % Relative standard deviation value is depicted in **Table 4**.

Table 4: Assay of Hydroxyzine

Label claim (mg)	Amount obtained (mg)	% Assay
25	24.98	99.92
25	24.81	99.24
25	25.17	100.68
Average	24.99	99.95
SD		0.7204
% RSD		0.7208

Evaluation of Greenness Profile

The results of greenness profile for the proposed methods were evaluated. The results of analytical scale is shown in **Table 5**, while the results agree metrics and GAPI is depicted in **Figure 12 and Figure 13**.

Table 5: Summary of Eco scale penalty points for the proposed method

Description	Penalty points	Total penalty Points	Score
Distilled water	0	0	100
Instrument	0		
Occupational hazard	0		
Waste	0		

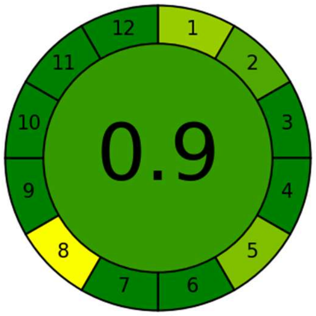

Figure 12: Agree metrics output for the proposed method

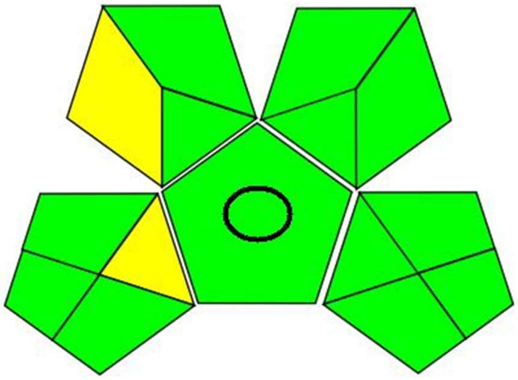

Figure 13: GAPI Pictogram for the proposed method

CONCLUSION:

Comparing the suggested method against the traditional UV-Visible Spectrophotometry for Hydroxyzine assay, it is more exact, accurate, repeatable, economical. It is evident from the greenness assessment tools that the method is ecofriendly. Hydroxyzine standard drug and tablet dosage form of Hydroxyzine quantified by the multivariate regression equation. According to the Quality Guidelines of ICH, this method has been validated and they are inside the range of the limits. This method was found simple and ecofriendly than complicated HPLC and HPTLC methods and it is used for the analysis of the sample of Hydroxyzine bulk drugs and pharmaceutical dosage forms.

ETHICAL STATEMENT

This study does not involve experiments on animals or human subjects.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article exists.

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