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MULTIVARIATE CALIBRATION BY UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF METADOXINE IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

This research aims to establish an UV-Visible spectroscopic technique to develop an easy, precise, sensible, and reproducible method for Metadoxine by applying a multivariate regression equation. Proposed technique depends on the equation of the linear regression performed by taking absorbance at five distinct wavelengths. Metadoxine's maximum absorbance was obtained at 324 nm. Graph obtained from concentration 14-26 µg 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 0.965733 and 0.349379. The assay value determined was between 99.78% - 99.90% w/w.

Keywords: Metadoxine, Hepatoprotective agent, UV spectrophotometry, Multivariate calibration, Assay, ICH guidelines

INTRODUCTION

Metadoxine is chemically referred as 5-oxo-L-proline compound with 5-hydroxy6-methyl pyridine-3,4-dimethanol. Structural formula and molecular weight was found to be $C_{13}H_{18}N_2O_6$ and 298.29 g mol^{-1} respectively **[1]**. Metadoxine is not official in any pharmacopeia. Metadoxine **(Figure 1)** is a synthetic antioxidant and possess antifibrotic properties, providing efficient protection against liver disease, alcohol intoxication, and hepatic stellate cell fibrosis. Pyroglutamic acid and vitamin B6 in a 1:1 ratio has been determined to function effectively together in Metadoxine.

Metadoxine is efficient in reducing glutathione which is essential for the redox equilibrium of the hepatic system and the entire body **[2]**. Literature review reveals various methods like Ultra-Visible spectroscopy (UV) **[3-8]**, High performance Liquid Chromatography (HPLC) **[9-13]**, High performance thin layer chromatography (HPTLC) **[14]**, for estimating Metadoxine.

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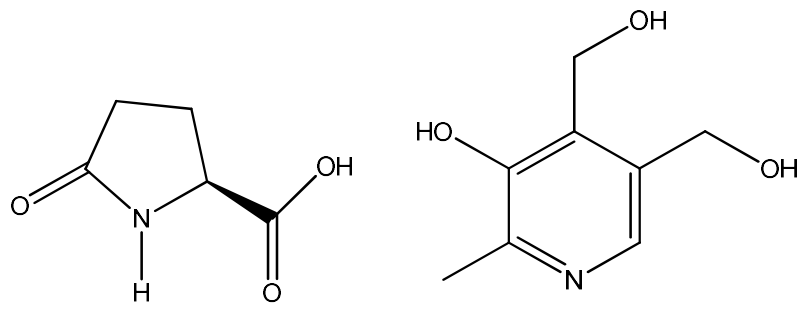

Figure 1: The chemical composition of metadoxine

The suggested technique provides higher confidence in results as it directly evaluates Metadoxine and has been attested with greater accuracy and precision than a classical UV-Visible assay. This technique is more cost-effective, direct, and rapid than other methods and can be used for bulk drugs and various dosage forms. A specific outcome was obtained from a multivariate standardization procedure and a dependent variable 'm' is obtained by the conversion from the outcome, this analytical technique provides excellent sensitivity, resolving power, expeditiousness, and economically efficient for a determined quantification of Metadoxine. Metadoxine (X) i.e., is the absorbance of an analyte, 7 different absorbances has to be scanned (λ = 318, 321, 324, 327, and 330nm); the following formula can then be applied for any preferred wavelength.

$A_{\lambda 318} = a\ X\ C_x + \ k_1$ ---------- (1)

$A_{\lambda 321} = b\ X\ C_x + \ k_2$ ---------- (2)

$A_{\lambda 324} = c\ X\ C_x + \ k_3$---------- (3)

$A_{\lambda 327} = d\ X\ C_x + \ k_4$---------- (4)

$A_{\lambda 330} = e\ X\ C_x + \ k_5$---------- (5)

Whereas absorbance of the analyte is denoted as A λ, the analyte's slope of the linear regression functions are a, b, c, d, and e; the corresponding five wavelengths indicates k1, k2, k3, k4, k5 of the intercepts, and the analyte's concentration is denoted as Cx. The selected five wavelengths equation (1-5) listed above summarised in the following:

$A_T = a\ X\ C_x + \ b\ X\ C_x + \ c\ X\ C_x +$

$d\ X\ C_x + \ e\ X\ C_x + \ K_T$---------- (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 AT and KT, respectively. The concentration of the analyte X is calculated using the formula below **[15–22]**.

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

MATERIALS AND METHODS

Chemicals and reagents

- Distilled water
- Metadoxine API was ex-gratis from Ideal Analytical Laboratory, Puducherry
- The marketed tablet formulation used was Viboliv manufactured by, Dr Reddy's Laboratories Ltd (Labelled to have 500 mg Metadoxine) purchased from a local market.

Instrumentation

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

Analytical method development

Solvent selection:

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

Standard stock solution

Metadoxine standard stock solution prepared by solubilizing 100 mg of standard drug into a 50 mL volumetric flask and distilled water is used to make up the volume. Aliquots of this solution (14 - 26µg/mL) were prepared and utilized for further analysis.

Determination of λ_{max}

The maximum absorbance of Metadoxine is determined from the solution prepared by dissolving the standard stock solution with water to 10 µg mL^{-1}. Prepared solution was measured in the range of 200 - 400 nm in the UV-Visible region. The maximum absorbance of Metadoxine obtained at 324 nm **(Figure 2)**. Obtained graph plot between the concentrations against absorbance gives a linear curve**.** The outcomes are examined around the spectrum range 324 nm, i.e., 318, 321, 324, 327, and 330 nm, for improving correlation and diminishing the oscillations of the instrument.

Sample solution preparation

Preparation of sample solution is done by taking twenty tablets of Metadoxine, precisely weighed and powdered. The weight equivalent to 100 mg was taken and transferred to a 100 mL standard flask, sonicated for 15 minutes, dissolved and made up to the mark with distilled water. This solution was then filtered, and from that sample solution is obtained that is used for further analysis.

Method Validation

This method has been validated for sensitivity, precision, accuracy, and linearity, accordance to the ICH guidelines **[23]**.

Linearity

From (14 – 26 µg/mL) various concentrations are prepared from standard stock solutions. These solutions were measured at a range of wavelengths 318, 321, 324, 327, and 330 nm to reduce instrumental fluctuations and improve the correlation **(Figure 3, Table 1)**. The graph is plotted as concentration against absorbance and standardizations were achieved.

Table 1: UV Calibration data at five distinct wavelengths

Concentration (µg mL^{-1})	Absorbance*				
	318 nm	321 nm	324 nm	327 nm	330 nm
14	0.269	0.284	0.307	0.289	0.268
16	0.316	0.325	0.334	0.328	0.310
18	0.351	0.372	0.376	0.371	0.349
20	0.391	0.409	0.416	0.409	0.388
22	0.429	0.452	0.459	0.449	0.426
24	0.467	0.495	0.501	0.490	0.467
26	0.506	0.536	0.545	0.536	0.513

[#]Average of 5 determinations; UV= Ultra violet

The limit of detection and quantification was determined by the sensitivity of the method using the formula below.

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

For measuring the intra-day and inter-day precision, the solution of 10 µg/mL was prepared and scanned for six times. The intra-day precision was measured within a day and the inter-day precision was measured in six various days.

Accuracy

The recovery study for recommended technique were concluded 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

Metadoxine amount present in the tablet calculated by measuring absorbance at 324 nm from the extracted tablet solution.

RESULTS AND DISCUSSION

The Metadoxine maximum absorbance obtained at 324 nm with water as the solvent as shown in **Figure 2**.

Within the concentration range between 14 - 26 µg/mL 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 obtained values of detection

and quantification of limit are 0.1627 and 0.4932 µg/ml., correspondingly. Hence the values come under validation parameters accordance to the ICH guidelines limitations.

Linearity

The linearity concentrations 14 -26 µg/mL were scanned between 220 - 400 nm as depicted in **Figure 3**, and the calibration curves are presented from **Figures 4 to 8**. The % relative standard deviation has low values that conclude technique is accurate and reliable is there for each wavelength. The calculation of detection and quantification of limit has been done and results were depicted in **Table 2**.

Precision

The recommended technique is unique, reliable and accurate hence it has low standard deviation values, correspondingly, the intra-day precision in addition to inter-day precision values obtained as 0.9657 and 0.3493. At each wavelength it should be within limits less than 2% **(Figure 9, 10)**.

Recovery

The Metadoxine % 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)**.

Assay:

The Metadoxine maximum absorbance was measured at 324 nm for the tablet formulation by using UV-Visible spectroscopy. Correspondingly, the quantity and assay percentages were obtained as 499.23 mg and 99.85 % w/w, further % Relative standard deviation values depicted at **Table 4**.

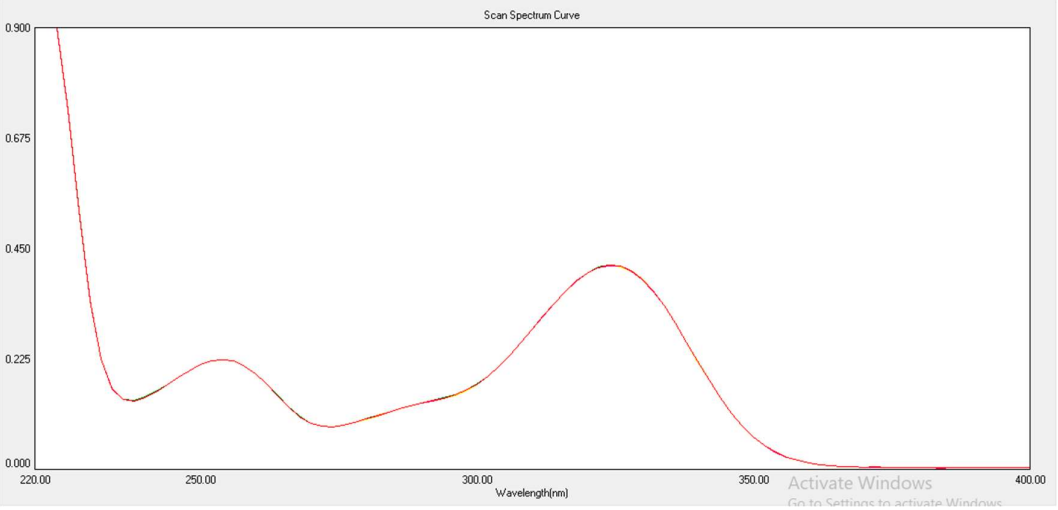

Figure 2: UV spectrum of Metadoxine (10 µg mL^{-1}), λ_{max} at 324 nm

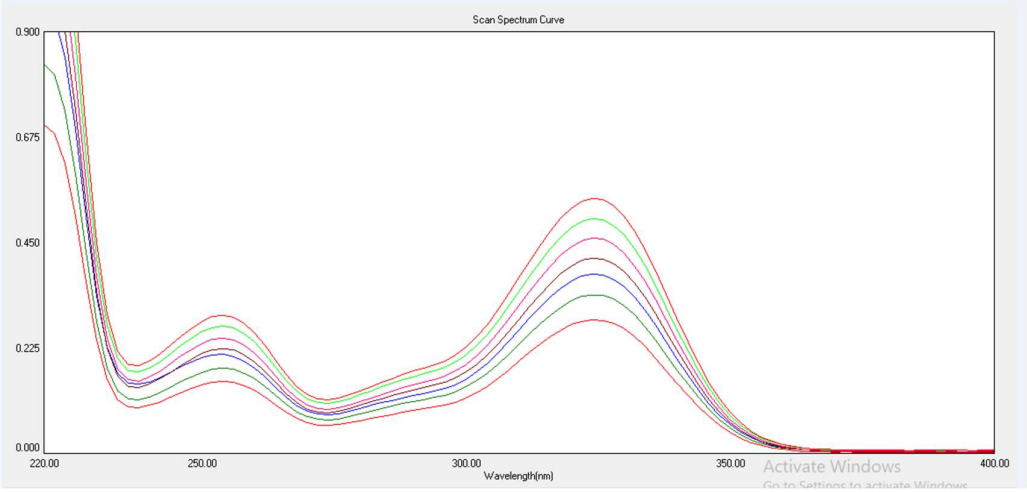

Figure 3: UV Spectrum of Metadoxine showing linearity

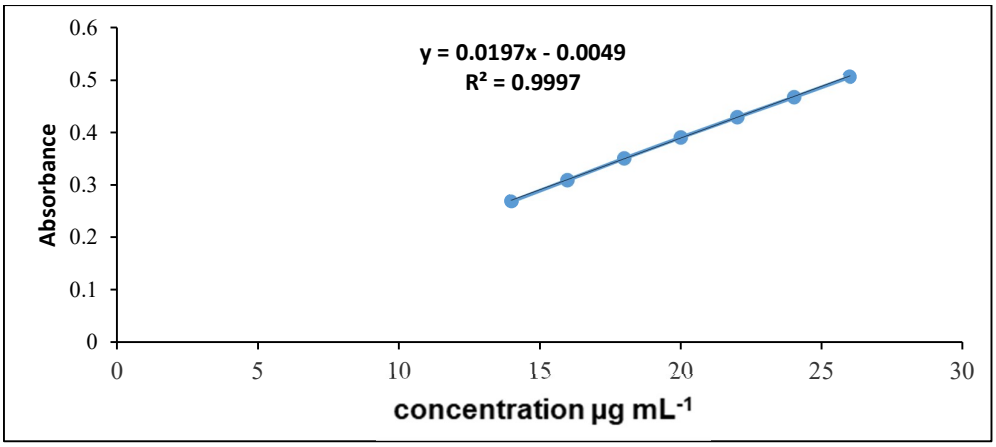

Figure 4: Calibration curve at 318 nm

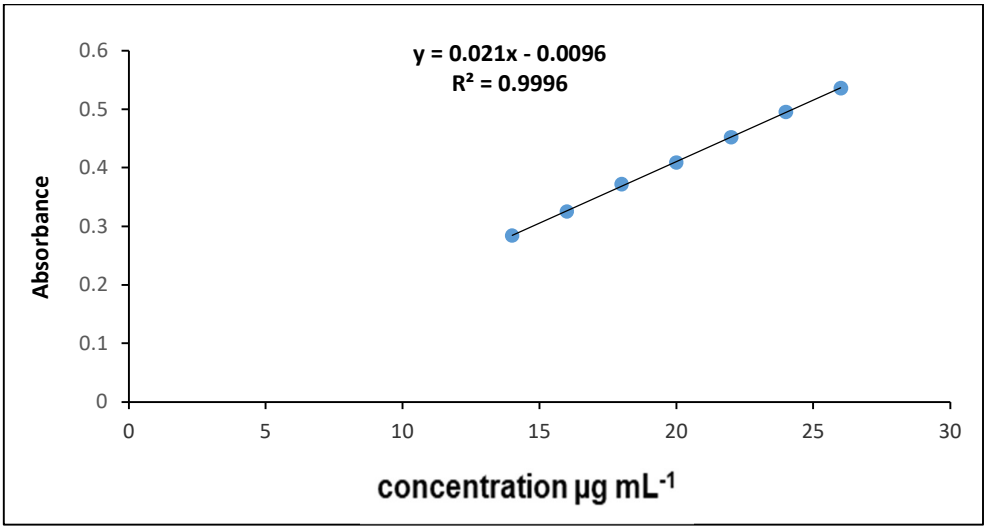

Figure 5: Calibration curve at 321 nm

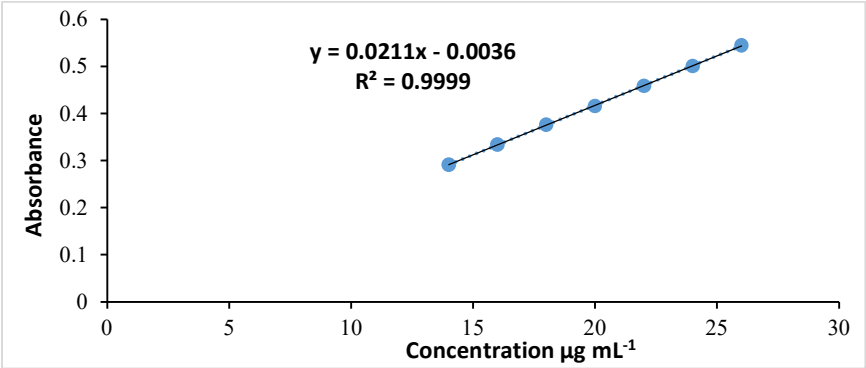

Figure 6: Calibration curve at 324 nm

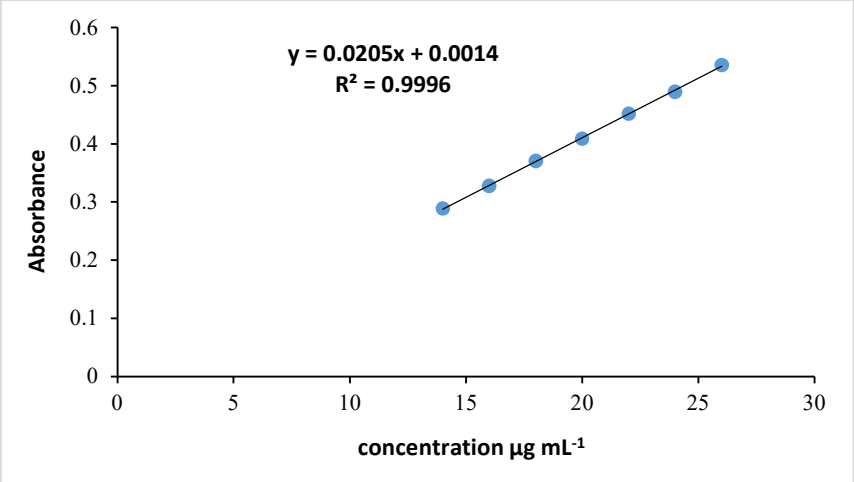

Figure 7: Calibration curve at 327 nm

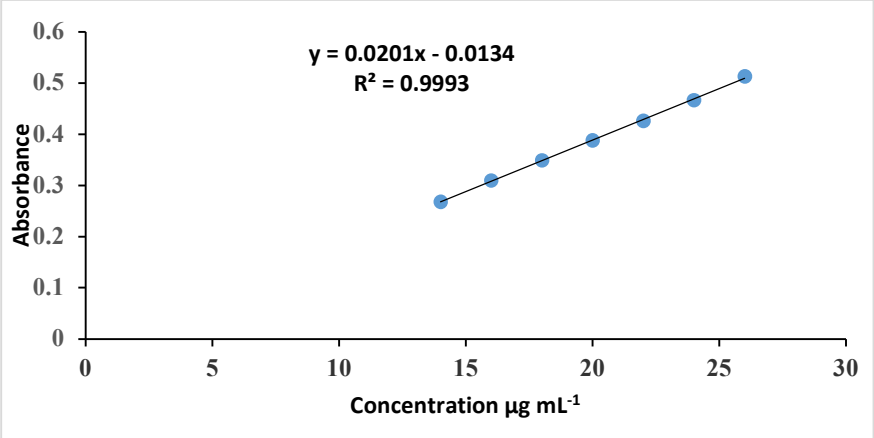

Figure 8: Calibration curve at 330 nm

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
318	y = 0.0197x - 0.0049	0.9997	0.2560	0.7759	0.3928
321	y = 0.021x - 0.0096	0.9996	0.2957	0.8963	0.4586
324	y = 0.0211x - 0.0036	0.9999	0.1627	0.4932	0.2487
327	y = 0.0205x + 0.0014	0.9996	0.3167	0.9597	0.4781
330	y = 0.0201x - 0.0134	0.9993	0.4029	1.2211	0.6316

*nm = nanometre; µg/mL = Microgram per millilitre

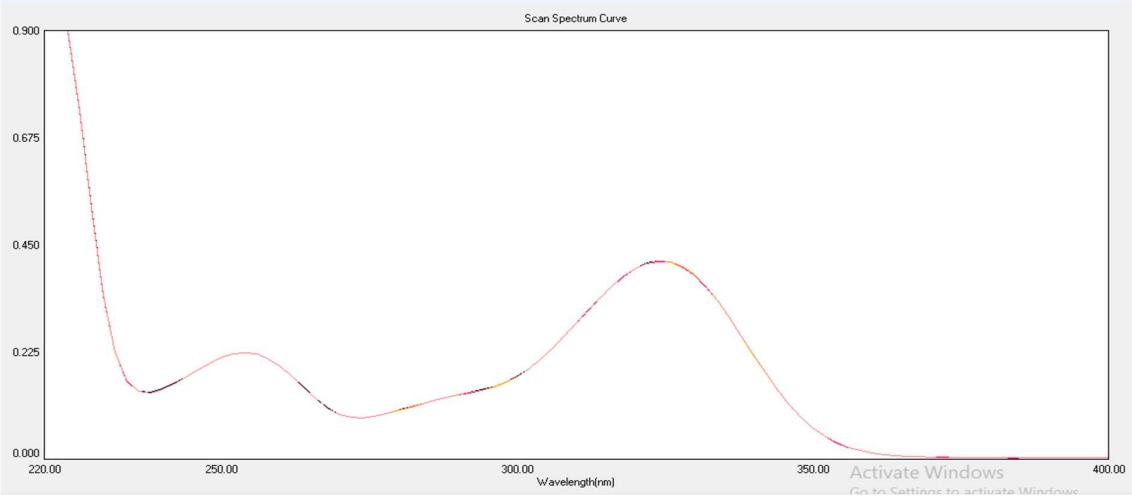

Figure 9: UV spectra showing intraday precision

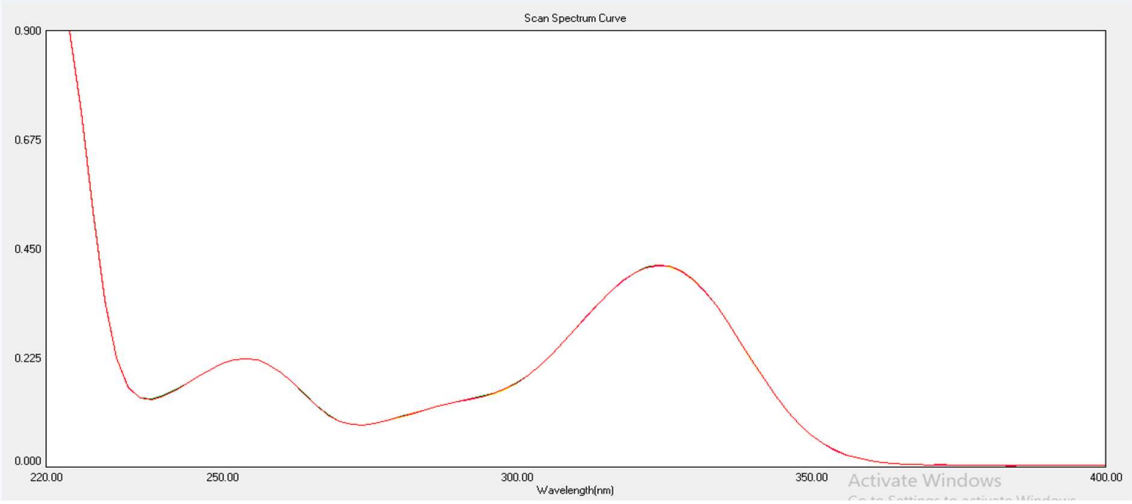

Figure 10: UV spectra showing interday precision

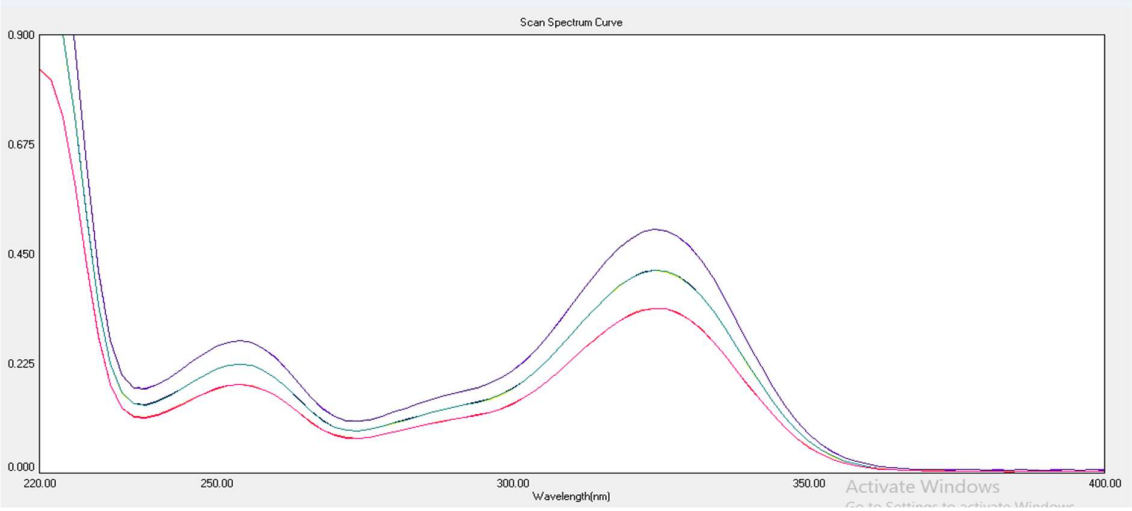

Figure 11: UV Spectrum showing accuracy of Metadoxine

Table 3: Recovery Studies

Wavelength (nm)	Amount present (µg mL^{-1})	Amount added (µg mL^{-1})	Absorbance	Amount recovered (µg mL^{-1})	% Recovery
318 nm	10	6	0.311	16.06	100.38
		10	0.391	19.98	99.90
		14	0.467	24.05	100.21
321 nm	10	6	0.396	15.99	99.94
		10	0.499	20.08	100.40
		14	0.587	24.09	100.38
324 nm	10	6	0.401	16.01	100.06
		10	0.509	19.94	99.70
		14	0.592	24.07	100.29
327 nm	10	6	0.397	15.89	99.31
		10	0.501	20.17	100.85
		14	0.588	24.13	100.54
330 nm	10	6	0.386	16.03	100.19
		10	0.493	20.06	100.30
		14	0.574	24.21	100.88

Table 4: Assay of Metadoxine

Label claim (mg)	Amount obtained (mg)	% Assay
500	498.89	99.78
500	499.5	99.90
500	499.29	99.86
Average	499.23	99.85
SD		0.0620
% RSD		0.0621

CONCLUSION:

The recommended technique is more accurate, precise, reproducible, cost-effective, and highly reliable than the conventional UV-Visible Spectrophotometry for Metadoxine assay. Metadoxine standard drug and tablet dosage form of Metadoxine tested usefully 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 than complicated HPLC and HPTLC methods and it is used for the analysis of the sample of Metadoxine 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.

FUNDING SOURCES

There is no funding to report.

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