Research Article

Method Development and Validation of Metadoxine in Marketed Tablet Formulation by UV Spectrophotometry Using Different Solvents

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ABSTRACT

Metadoxine is an antioxidant and hepatoprotective drug with major application in the control of alcohol-induced liver diseases and conditions related to oxidative stress. The present research is about the development and validation of a precise, cost-effective, and straight forward UV spectrophotometric method for the quantification of Metadoxine in commercial tablet formulations by employing different solvents such as alkaline water, distilled water, and methanol. Method development entailed establishing the wavelength maxima (λmax) of Metadoxine in all three solvents, and it was established at 218 nm for alkaline water and 284 nm for methanol and distilled water. Linearity was noted with concentration ranges of 6–18 µg/mL (alkaline water), 6–14 µg/mL (distilled water), and 5–30 µg/mL (methanol), with a correlation coefficient (r^2) of not more than 0.999, signifying excellent linearity. Validation of the procedure adhered to ICH Q2 (R1) regulations, including parameters like precision, accuracy, robustness, and sensitivity. The outcomes proved recovery percentages between 96–99%, while the %RSD was below 2%, establishing the reproducibility of the procedure. In addition, the employment of alkaline water as a solvent is an environmentally friendly and cost-effective approach over traditional organic solvents such as methanol, supporting sustainable laboratory practice. The method developed is thus appropriate for regular quality control analysis of Metadoxine in drug products.

Keywords: Metadoxine, Method development, UV-Visible Spectrophotometer, Method Validation, Alkaline water, Distilled water, Methanol.
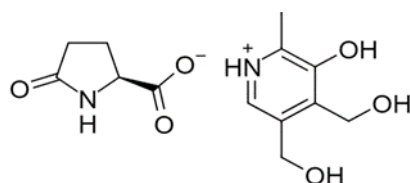
INTRODUCTION

Metadoxine (pyridoxine L-2-pyrrolidone-5-carboxylate) is a unique compound that combines the beneficial properties of pyridoxine (vitamin B6) with the antioxidant and detoxification effects of pyroglutamic acid[1-3]. It is widely used in the treatment of alcohol-induced liver diseases**,** alcoholic fatty liver disease, and hepatic encephalopathy[4-5]. Traditional methods for the estimation of Metadoxine include high-performance liquid chromatography (HPLC)**,** colorimetric assays, and titrimetric methods [6-7]. While these techniques are sensitive and reliable, they require expensive equipment, extended analysis time, and hazardous organic solvents[8-9]**.** In contrast, UV spectrophotometry offers a simple, rapid, and cost-effective alternative for routine quality control analysis[10-11]. The current study is novel due to the use of alkaline water as a solvent**,** which provides an eco-friendly and sustainable alternative to organic solvents like methanol[12-13].

Drug Profile of Metadoxine

Drug Name: Metadoxine

Structure:

Category: Hepatoprotective, Antioxidant

Molecular Formula: $C_{13}H_{18}N_2O_6$

Molecular Weight: 298.30 g/mol

Melting Point: 102–104°C

IUPAC Name: 5-Oxo-L-proline-4, 5-bis (hydroxymethyl)-2-methylpyridin-3-ol

Appearance: White or off-white crystalline powder

Solubility: Freely soluble in water and methanol

Mechanism of Action: Enhances ethanol clearance, reduces oxidative stress

Uses: Treatment of alcoholic fatty liver disease, potential neuroprotective effects[14-15]

MATERIALS AND METHODS

Chemicals and solvents

Metadoxine pure drug was obtained as a gift sample from Suven Pharmaceuticals Ltd., Hyderabad. Tablets containing 500 mg of Metadoxine were gifted by Leads Pharma Pvt. Ltd., Hyderabad. Alkaline water (EVOCUS), distilled water and methanol are of analytical grade. Double-distilled water was used for all solution preparations.

Equipment and instruments

1. UV-Visible Spectrophotometer- Shimadzu 1800 Double Beam UV-Visible Spectrophotometer**:** 10 mm quartz cuvettes

2. Analytical Balance- Shimadzu ATX124: 0.1 mg sensitivity

3. Sonicator- PCI Ultrasonic

4. Glassware- Class-A volumetric flasks, pipettes, and beakers: High accuracy laboratory-grade glassware

Preparation of solutions

Preparation of Stock Solution (1000 µg/mL)

Accurately weighed 100 mg of Metadoxine. Transferred the drug to a 100 mL volumetric flask and added 10 mL of solvent (alkaline water/distilled water/methanol). It was sonicated for 10 minutes to dissolve the drug completely. The volume was made up to 100 mL with the same solvent to obtain 1000 µg/mL stock solution.

Preparation of Working Standard

From the stock solution, serial dilutions were prepared to obtain concentrations in the range of 5–30 µg/mL (methanol), 6–14 µg/mL (distilled water), and 6–18 µg/mL (alkaline water).

Determination of λ$_{max}$

A 10 µg/mL solution of Metadoxine was prepared in each solvent. The solutions were scanned over the range of 200–400 nm using a UV spectrophotometer. λmax values were obtained at 218 nm for alkaline water and for methanol and distilled water λ$_{max}$ was obtained at 284 nm.
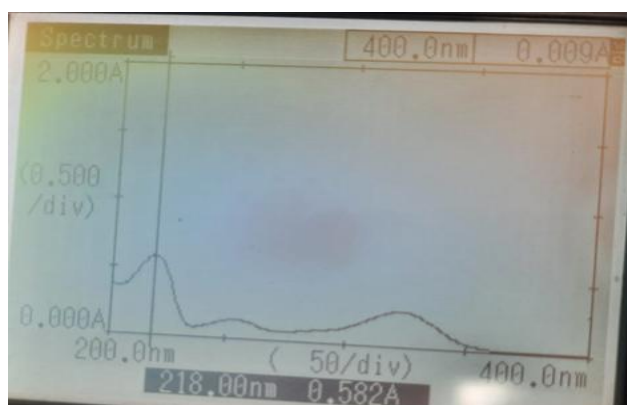
Sample Preparation from Marketed Tablets

Twenty tablets were weighed and finely powdered. An amount of powder equivalent to 100 mg of Metadoxine was accurately weighed. The powder was dissolved in 10 mL of solvent. Sonicated for 15 minutes and filtered through Whatman filter paper. The filtrate was diluted to 100 mL to prepare the sample solution (1000 µg/mL). Further dilutions were made to bring the solution into the linearity range.

RESULTS AND DISCUSSION

Wavelength Selection

Wavelength scans confirmed sharp absorption peaks at 218nm as shown in Figure 1.

Figure 1: λ$_{max}$ of Metadoxine in Alkaline water (λ$_{max}$ = 218 nm)

Validation Parameters

The developed UV spectrophotometric method was validated as per ICH Q2 (R1) guidelines. The parameters evaluated include:
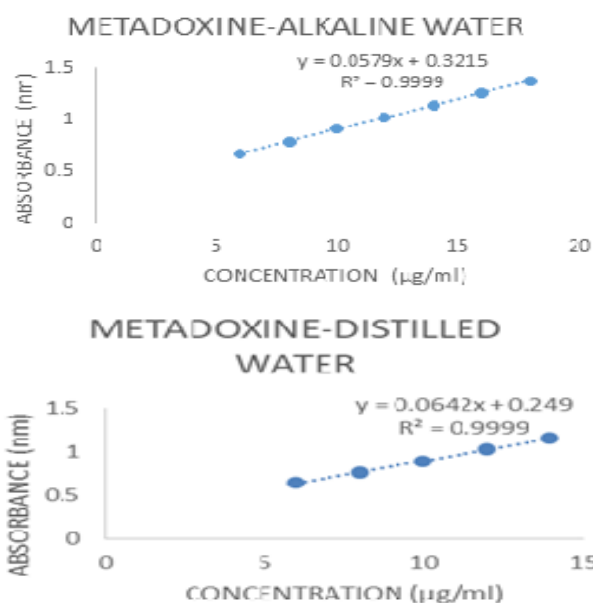
Linearity and Range

Linearity was assessed by analyzing standard solutions of Metadoxine across the ranges of 6-18 µg/mL for alkaline water, 6-14µg/ml for distilled water and 5-30µg/mL for methanol (Table 1). Figure 2 depicts the Calibration graphs of Metadoxine with different solvents.

Table 1: Results of Linearity

Solvents	Concentration (µg/ml)	Absorbance (nm)
Alkaline Water	6	0.665
	8	0.785
	10	0.902
	12	1.019
	14	1.135
	16	1.214
	18	1.361
Distilled water	6	0.636
	8	0.762
	10	0.888
	12	1.020
	14	1.149
Methanol	5	0.131
	10	0.224
	15	0.322
	20	0.416
	25	0.614
	30	0.759

Linearity Graph

METADOXINE-ALKALINE WATER

$y = 0.0579x + 0.3215$
$R^2 = 0.9999$

METADOXINE-DISTILLED WATER
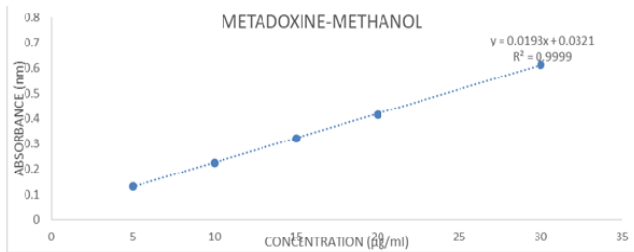
$y = 0.0642x + 0.249$
$R^2 = 0.9999$

Figure 2: Calibration graph for Metadoxine-Alkaline Water, distilled water and methanol

Precision

Precision was determined by measuring absorbance values of a 10 µg/mL solution for alkaline water and distilled water and 25 µg/mL for methanol are taken in six replicates, both intraday and inter day. Interday precision and intraday precision results are presented in table 2.

Accuracy

Accuracy was evaluated by spiking known amounts of standard drug at 50%, 100%, and 150% levels to pre-analyzed samples. Table 3 shows the results of accuracy.

Table 2: Intraday precision Data and Inter day Precision Data

Intraday precision										
Time	Conc (µg/ml)	Absorbance						Mean	S.D	%RSD
Alkaline water	10	0.976	0.978	0.977	0.976	0.977	0.979	0.977	0.0010	0.10
Distilled water	10	0.793	0.795	0.796	0.795	0.794	0.794	0.794	0.0009	0.12
Methanol	25	0.622	0.622	0.622	0.622	0.622	0.622	0.622	0	0
Inter day Precision										
Day	Solvents	Absorbance						Mean±SD		%RSD
1	Alkaline water	0.929	0.929	0.929	0.929	0.929	0.930	0.929±0.0003		0.04
	Distilled water	0.787	0.787	0.787	0.787	0.787	0.786	0.786±0.0003		0.04
	Methanol	0.639	0.640	0.639	0.643	0.639	0.639	0.639±0.001		0.07
2	Alkaline water	0.983	0.985	0.987	0.984	0.985	0.985	0.984±0.001		0.12
	Distilled water	0.799	0.799	0.799	0.799	0.799	0.799	0.799±0		0
	Methanol	1.21	1.21	1.21	1.21	1.21	1.21	1.21±0		0
3	Alkaline water	0.991	0.992	0.990	0.990	0.991	0.993	0.991±0.001		0.10
	Distilled water	0.796	0.796	0.797	0.797	0.797	0.797	0.796±0.0004		0.05
	Methanol	1.21	1.22	1.22	1.22	1.23	1.22	1.22±0.005		0.47

Table 3: Accuracy data

Solvents	% Level	% Recovery	Mean % Recovery
Alkaline water	50	96.97 97.5 98.5	97.6
	100	99.11 98.5 98.2	98.6
	150	99.6 99.4 99.11	99.37
Distilled water	50	96.91 96.5 97.5	96.97
	100	98.01 98.22 98.73	98.32
	150	98.07 97.95 99.15	98.39
Methanol	50	97.97 99.58 98.98	98.84
	100	98.12 98.58 98.96	98.55
	150	98.65 97.14 98.13	97.97

LOD and LOQ

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The sensitivity of the developed method was assessed by calculating the LOD and LOQ using the following equations:

$$LOD = 3.3 \times \sigma / S$$

$$LOQ = 10 \times \sigma / S$$

Where:

σ = Standard deviation of the response

S = Slope of the calibration curve

The low LOD and LOQ values indicate that the developed UV spectrophotometric method is sensitive enough to detect and quantify low levels of Metadoxine. Table 4 shows the LOD and LOQ data.

Table 4: LOD and LOQ results

Solvent	LOD (µg/mL)	LOQ (µg/mL)
Alkaline water	0.0608	0.1843
Distilled water	0.0492	0.1491
Methanol	0.0527	0.1586

Robustness

Robustness was evaluated by making small deliberate changes in analytical parameters, such as Wavelength variation by ±1 nm from 218 nm, solvent ratio variations by minor changes in dilution and time stability of samples by

analyzing after 30, 60, and 90 minutes. Robustness data is shown in table 5.

Table 5: Robustness data for alkaline and distilled water.

Solvents	Wavelength (nm)	Absorbance (10 µg/mL)	%RSD
Alkaline water	217	0.542	0.39
	218	0.541	0.32
	219	0.543	0.35
Distilled water	217	0.793	0.12
	218	0.795	0.12
	219	0.796	0.13
Methanol	283	0.622	0.2
	284	0.622	0.1
	285	0.622	0.12

The %RSD values were within acceptable limits (<2%), confirming that slight changes in analytical parameters do not significantly affect the results.

Assay

The developed UV spectrophotometric method was applied to estimate Metadoxine in marketed tablet formulations. The results were consistent with the labelled claim.

Table 6: Assay results

Sample	Labeled Claim (mg)	Amount Found (mg)	% Assay
Tablet 1	500	495.6	99.1
Tablet 2	500	496.8	99.3
Tablet 3	500	494.2	98.8

Average Assay: 99.0%

Acceptance Criteria: 98–102%

DISCUSSION

The results of the method validation confirm that the UV spectrophotometric method developed for Metadoxine is both reliable and reproducible. The linearity range was established for all solvents, with correlation coefficients ($r^2 \geq 0.999$), which confirms the linearity range. The % recovery (96–99%) indicates the method's accuracy and its ability to measure the analyte without interference from excipients. Precision studies (intraday and interday) revealed %RSD values less than 2%, fulfilling ICH Q2 (R1) guidelines. One of the notable findings of this study is the successful use of alkaline water as a solvent, which reduces the dependence on hazardous organic solvents like methanol, lowers the cost of analysis and aligns with green analytical chemistry principles by using an eco-friendly and sustainable approach. When compared with other studies involving HPLC or traditional UV methods, the proposed method is faster, simpler, and does not require complex instrumentation.

CONCLUSION

The present study successfully developed and validated a UV spectrophotometric method for the estimation of Metadoxine in marketed tablet formulations using alkaline water, distilled water, and methanol as solvents. The eco-friendly approach using alkaline water offers an environmentally sustainable and cost-effective alternative to traditional solvents like methanol. The method is suitable for routine quality control of Metadoxine in pharmaceutical industries.

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