NEW SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF METADOXINE IN BULK AND PHARMACEUTICAL FORMULATIONS BASED ON REDOX AND OXIDATIVE COUPLING REACTIONS

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ABSTRACT
Metadoxine (MTD) is a synthetic antioxidant, providing strong antioxidant protection. It is useful in preventing the damage produced in early stages of liver disease. It is used in alcohol intoxication. In hepatic stellate cells it prevents the collagen synthesis & reduces fibrosis. Two simple and sensitive spectrophotometric methods (Method A and Method B) were developed for the estimation of MTD in pharmaceutical formulations. Method A is based on redox reactions of MTD with Folin-Ciocalteu reagent (FC reagent) to form a stable blue coloured chromogen, which can be estimated at 660nm. Method B is based on the oxidation of MTD with Fe (III) under controlled experimental conditions followed by complex formation between the reduced form of Fe III (Fe II) with 1,10-phenanthroline (PTL) to give colored complex which can be measured at 460nm. Method A obeys Beer’s law in the concentration range of 5-30 μg/mL and Method B in concentration of 4-24 μg/mL. The common excipients usually present in dosage forms do not interfere with any of the proposed Methods. The methods were validated for use in routine quality control of MTD in pharmaceutical formulations.
Key words: Metadoxine, Folin-Ciocalteu reagent, 1,10-phenanthroline, Spectrophotometric determination.

INTRODUCTION
Metadoxine (MTD)1,2, a 5-oxo-L-proline compound with 5-hydroxy 6-methyl pyridine-3,4-dimethanol, is a synthetic antioxidant. It is useful in preventing the damage produced in early stages of liver disease as it prevents the redox imbalance of the hepatocytes &TNF-a indication, one of the earliest event in hepatic damage. It is used in
alcohol intoxication \cite{3,4}. In hepatic stellate cells it prevents the collagen synthesis & reduces fibrosis it acts as an antifibrotic agent.

The literature survey reveals the availability of few spectrophotometric \cite{5,6,7}, HPLC \cite{8,9,10} and a HPTLC\cite{11} method for the determination of MTD in pharmaceutical formualtions.

In the present investigation we developed two spectrophotometric methods for the determination of the drug in pharmaceutical formulations based on the drugs redox reaction with Folin-Ciocalteu reagent (FC reagent)\cite{12,13} - Method A and Oxidative coupling reaction with 1,10-phenanthroline (PTL) - Method B.

![Structure of MTD](image)

**MATERIALS AND METHODS**

**INSTRUMENTATION:**

A Systronics Double beam UV- visible spectrophotometer 2201 with 1 cm matched quartz cells was used for all spectral and absorbance measurements.

**REAGENTS PREPARATION:**

**Method A :**

- **FC reagent solution** (Loba, 0.67 N): Prepared by diluting three times of commercially available Folin-Ciocalteu reagent (2N) with distilled water.
- **NaOH solution** (Merck, 4% w/v, 1.89 x 10^{-1} M): Prepared by dissolving 4.0 gm of sodium hydroxide in 100 mL of distilled water.

**Method B :**

- **PTL solution** (Qualigens, 0.198% w/v, 1.0 x 10^{-2} M): Prepared by dissolving 198 mg of 10-phenanthroline in 100 mL of 0.1N hydrochloric acid.
• FeCl₃ stock solution (CDH, 0.162 % w/v, 1M) (3.3 x 10⁻³ M): About 162 mg of anhydrous ferric chloride was accurately weighed and dissolved in 100 mL of distilled water. 33.3 mL of above stock solution was further diluted to 100 mL with water. 50 mL of above stock solution was further diluted to 100 mL with water.

• OPA solution (CDH, 2.0 x 10⁻¹ M): 1.3 mL of orthophosphoric acid was diluted 100 mL with distilled water.

**PREPARATION OF STANDARD DRUG SOLUTIONS:**

About 500 mg of MTD was accurately weighed and dissolved in 100 mL of water containing volumetric flask to get a stock solution of 5mg/mL. 1mL of the stock solution was further diluted to 10 mL of volumetric flask to get a working standard solution of concentration 500 µg/mL for method A.

For method B, 1mL of the stock solution was further diluted to 25 mL of volumetric flask to get a working standard solution of concentration 200 µg/mL.

**SAMPLE PREPARATION:**

The content of twenty Tablets was transferred to a mortar. The Tablet powder was mixed and thoroughly ground with mortar. From this Tablets powder equivalent to 500 mg of MTD was taken and extracted into 100 mL of water then it was appropriately diluted with the same solvent and used for method A and B.

**PROCEDURE FOR ESTIMATION:**

**Method A:**

Into a series of 10 mL volumetric flasks, standard solution (500µg/mL) of MTD in the concentration range of 50 – 300µg (0.1mL – 0.6 mL) were transferred. Then 3.0 mL of NaOH solution, 1.0 mL of FC reagent were successively added and kept aside for 5 min. The volume was made up to 10 mL with water. The absorbance was measured at 660 nm against reagent blank. The amount of MTD was deduced from its Beer-Lambert’s plot.

**Methods B:**

Aliquots of standard MTD solution (200µg/mL) containing 40 to 240µg were transferred into a series of 10 mL volumetric flasks and 1.0 mL of 0.003 M ferric chloride was added to each flask. Then 1.0 mL of PTL solution was added to all flasks and the
volumes in all volumetric flasks were equalized with water. The contents were gently boiled for 35 min, the flasks were cooled to room temperature and 2.0 mL of OPA was added to all and final volume of all volumetric flasks was brought to 10 mL with water. The absorbance was measured at 460 nm against corresponding reagent blanks. The amount of MTD in sample was estimated from corresponding calibration graph.

RESULTS AND DISCUSSION

Method A:

The color formation by FC reagent with MTD may be explained in the following manner based on the analogy with the reports of earlier workers. The mixed acids in FC reagent preparation involve the following chemical species.

\[ 3H_2O \cdot P_2O_5 \cdot 13WO_3 \cdot 5MoO_3 \cdot 10H_2O \]

\[ 3H_2O \cdot P_2O_5 \cdot 14WO_3 \cdot 4MoO_3 \cdot 10H_2O \]

MTD probably effects a reduction of 1, 2 or 3 oxygen atoms from the tungstate and/or molybdate, thereby producing one or more of several possible reduced species, which have characteristic intense blue color.

Method B:

MTD exhibits reducing property due to the presence of functional moieties (one or more) vulnerable to oxidation selectively with oxidizing agents such as Fe (III) under controlled experimental conditions. When treated with known excess of oxidant, MTD undergoes oxidation, giving products of oxidation (inclusive of reduced form of oxidant, Fe (II) from Fe (III), besides unreacted oxidant. It is possible to estimate the drug content colorimetrically, which is equivalent to either the reacted oxidant or reduced form of oxidant formed. The reduced form of Fe III (Fe II) has a tendency to give colored complex on treatment with PTL(M_B).

The first step in the methods mentioned above is the oxidation of MTD with the oxidant.

\[
\text{MTD} + \text{Fe (III)} \rightarrow \text{Oxidation products} + \text{Fe (II)} + \text{Fe (III)}
\]

(Excess) (Reduced form (Unreacted)
of Oxidant)
In this method, as Fe (III) interferes, even though to a little extent in the determination of Fe (II), the reactivity of the interfering entity has to be made insignificant by complexing it with o-phosphoric acid.

\[ \text{Fe (III)} + \text{o-phosphoric acid} \rightarrow \text{Complex (unreactive)} \]

The second step concerns with the estimation of the reduced form of oxidant with chromogenic agent i.e, PTL. The complex formation for these methods is shown in scheme no. 1.

Interference studies were conducted to see the influence of excipients with the proposed methods. The common excipients usually present in dosage forms do not interfere in the proposed method A and method B. The optical characteristics, regression analysis data and precision of the methods are presented in table no 1. The accuracy of the methods was evaluated by estimating the amount of MTD in previously analyzed samples to which known amounts of MTD was spiked. The accuracy of the methods was also confirmed by comparison of the results obtained by proposed and reference methods. The results of accuracy were given in table-2. Some of the commercially available formulations were procured from the local market and analyzed by the developed methods and the results comply with the labeled claim (table-2).
Table 1: Optical characteristics and regression analysis parameters precision and accuracy of the proposed methods for MTD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method A</th>
<th>Method B</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>660</td>
<td>460</td>
</tr>
<tr>
<td>Beer’s law limits (μg/mL)</td>
<td>5-30</td>
<td>4-24</td>
</tr>
<tr>
<td>Molar absorptivity (L. mole$^{-1}$ cm$^{-1}$)</td>
<td>$6.26 \times 10^3$</td>
<td>$7.97 \times 10^4$</td>
</tr>
<tr>
<td>Detection limits (μg/mL)</td>
<td>0.0571</td>
<td>0.0312</td>
</tr>
<tr>
<td>Sandell’s sensitivity (μg/cm$^2$/0.001 absorbance unit)</td>
<td>0.047</td>
<td>0.0373</td>
</tr>
<tr>
<td>Optimum photometric range</td>
<td>7.5-25</td>
<td>8-22</td>
</tr>
<tr>
<td>Regression equation ($Y = a + bc$):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.0193</td>
<td>0.0265</td>
</tr>
<tr>
<td>Standard deviation of slope (Sb)</td>
<td>0.000375</td>
<td>0.000115</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>0.0126</td>
<td>-0.00075</td>
</tr>
<tr>
<td>Standard deviation of intercept (Sa)</td>
<td>$6.76 \times 10^{-4}$</td>
<td>$1.65 \times 10^{-4}$</td>
</tr>
<tr>
<td>Standard error of estimation($S_e$)</td>
<td>$9.92 \times 10^{-4}$</td>
<td>$24.2 \times 10^{-4}$</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9997</td>
<td>0.9999</td>
</tr>
<tr>
<td>% Relative standard deviation*</td>
<td>0.0261</td>
<td>0.380</td>
</tr>
<tr>
<td>% Range of Error (Confidence limits)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05 level</td>
<td>0.897</td>
<td>0.90</td>
</tr>
<tr>
<td>0.01 level</td>
<td>1.121</td>
<td>1.406</td>
</tr>
<tr>
<td>% Error in bulk samples**</td>
<td>0.33</td>
<td>-0.51</td>
</tr>
</tbody>
</table>

* Average of six determinations

** Average of three determinations.
Table 2: Assay and recovery of in dosage forms

<table>
<thead>
<tr>
<th>Method</th>
<th>Pharmaceutical Formulation</th>
<th>Labelled Amount (mg)</th>
<th>Proposed Method</th>
<th>Found by reference method ± S.D</th>
<th>% recovery by proposed methods** ± S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Amount found* (mg) ± S.D</td>
<td>t (value)</td>
<td>F (Value)</td>
</tr>
<tr>
<td>Method A</td>
<td>Tablet</td>
<td>500</td>
<td>499 ± 0.012</td>
<td>0.652</td>
<td>2.125</td>
</tr>
<tr>
<td>Method B</td>
<td>Tablet</td>
<td>500</td>
<td>500 ± 0.011</td>
<td>0.572</td>
<td>1.893</td>
</tr>
</tbody>
</table>

* Average ± standard deviation of six determinations, the t and F- values refer to comparison of the proposed method with reference method. Theoretical values at 95 % confidence limits t = 2.571 and F = 5.05.

** Average of five determinations

CONCLUSION

The proposed methods are economic, simple, sensitive, reproducible and accurate and can be used for the routine analysis of MTD in bulk as well as in its pharmaceutical preparations.

ACKNOWLEDGMENTS

The authors are thankful to Siddhartha Academy of General and Technical education, Vijayawada for providing facilities to carry out the present work.

REFERENCES


