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NEW SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF METADOXINE IN BULK AND PHARMACEUTICAL FORMULATIONS BASED ON OXIDATIVE COUPLING AND CHARGE-TRANSFER COMPLEX FORMATION 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 as it prevents the redox imbalance of the hepatocytes and TNF- one of the earliest event in hepatic damage. It is used in alcohol intoxication. In hepatic stellate cells, it prevents the collagen synthesis and reduces fibrosis. Three simple and sensitive spectrophotometric methods (Method A, method B and method C) were developed for the estimation of MTD in pharmaceutical formulations. Method A and method B are based on oxidative coupling of MTD with 3-methyl-2-benzothiazolinone hydrazone reagent in the presence of oxidizing agents like Fe (III) and Ce (IV) to form a stable violet coloured chromogen, which can be estimated at 580 nm. Method C is based on the charge-transfer complex formation between metol and MTD to produce a chromophore, which can be measured at 500 nm.

Method A and method B obey Beer's law in the concentration range of 2-10 µg/mL and method C in concentration of 2-12 µg/mL. The common excipients usually present in dosage forms do not interfere with any of the proposed methods. The optical characteristics, regression analysis data and precision of the methods were also calculated. 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 methods were validated for use in routine quality control of MTD in pharmaceutical formulations.

Key words: Metadoxine, Spectrophotometry, Development, Validation, 3-Methyl-2-benzothiazolinone hydrazone reagent (MBTH), Metol.

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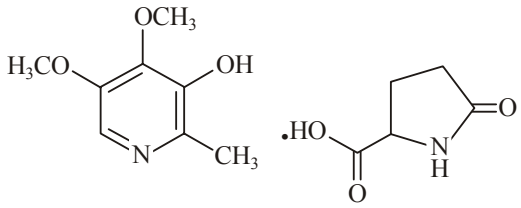
INTRODUCTION

Metadoxine (MTD), 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 and TNF-a indication, one of the earliest event in hepatic damage. It is used in alcohol intoxication[1-4]. In hepatic stellate cells, it prevents the collagen synthesis and reduces fibrosis. It acts as an antifibrotic agent.

The literature survey reveals the availability of spectrophotometric[5], HPLC[6] and HPTLC[7] method for the determination of MTD in pharmaceutical formullations.

In the present investigation, we have developed two spectrophotometric methods based on the drugs oxidative coupling reaction MBTH[8] (Method A and method B) and one method based on formation of a charge-transfer complex with metol[9] (Method C)



Structure of MTD

EXPERIMENTAL

Instrumentation

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

Reagents preparation

Method A and Method B:

3-Methyl-2-benzothiazoloinone hydrazone) solution (0.2 % w/v): This solution was prepared by dissolving 200 mg of 3-methyl-2-benzothiazolinone hydrazone in 100 mL of distilled water.

Ferric chloride solution (0.1% w/v): It was prepared by dissolving 100 mg of ferric chloride in 100 mL of distilled water.

CAS solution (Merck, 0.1%, 6.17 x 10^{-3} M): It was prepared by dissolving 100 mg of cerric ammonium sulphate in 100 mL of distilled water.

Method C:

Metol solution (Qualigens 0.2% w/v, 5.81 x 10^{-3} M): This solution was prepared by dissolving 200 mg of p-N-methylaminophenol in 100 mL of distilled water.

KIO_3 solution (Merck, 0.43%, 2.0 x 10^{-2} M): It was prepared by dissolving 430 mg of potassium iodate in 100 mL of distilled water.

pH 3.1 buffer solution: The buffer (3.1) was prepared by diluting a mixture of 50 mL of 0.2 M potassium hydrogen phthalate and 19 mL of 0.2N HCl to 200 mL with water and the pH was adjusted to 3.1.

Preparation of standard drug solutions

For methods, A and B: About 500 mg of MTD was accurately weighed and dissolved in 100 mL of water in volumetric flask to get a stock solution of 5 mg/mL. 1 mL of the stock solution was further diluted to 100 mL to get a working standard solution of concentration 50 μg/mL for method A and B.

For method C: About 500 mg of MTD was accurately weighed and dissolved in 100 mL of water in a volumetric flask to get a stock solution of 5 mg/mL. 1 mL of the stock solution was further diluted to 50 mL to get a working standard solution of concentration 100 μg/mL for method C.

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. Later, this solution was further diluted to get absorbance values within the calibration curve range.

Procedure for estimation

Methods A and B: Aliquots of standard MTD solution (50 μg/mL) ranging from 0.4 to 2 mL were transferred into a series of 10 mL volumetric flasks. To each flask, 2.0 mL of ferric chloride solution and 1.0 mL of CAS were added for method A and B, respectively. 1.0 mL of MBTH solution was added to each flask and allowed to stand at room temperature

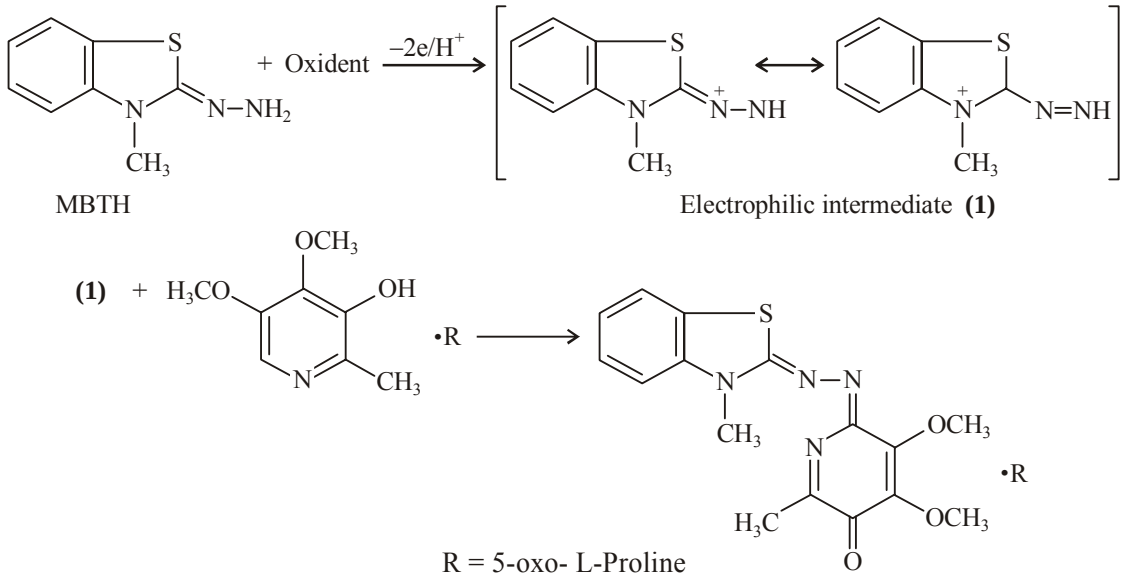
for 15 min. The final volume was adjusted to 10.0 mL with water and the absorbance was measured at 580 nm against a reagent blank. The amount of MTD in sample was calculated from the Beer-Lambert's plot.

Method C: Into a series of 25 mL volumetric flasks, 15 mL of pH 3.1 buffer, 1.0 mL of 0.02 M KIO_3 solution and 2.0 mL of 0.2% metol solution were successively placed. To this, aliquots of standard solution (100 µg/mL) of MTD in the range of 0.5-3.0 mL were added. The final volume in each flask was made up to the mark with distilled water. The absorbance of the solutions was measured at 500 nm after 20 min. against the reagent blank. The amount of MTD in test was computed from the corresponding Beer-Lambert's plot.

RESULTS AND DISCUSSION

Methods A and B

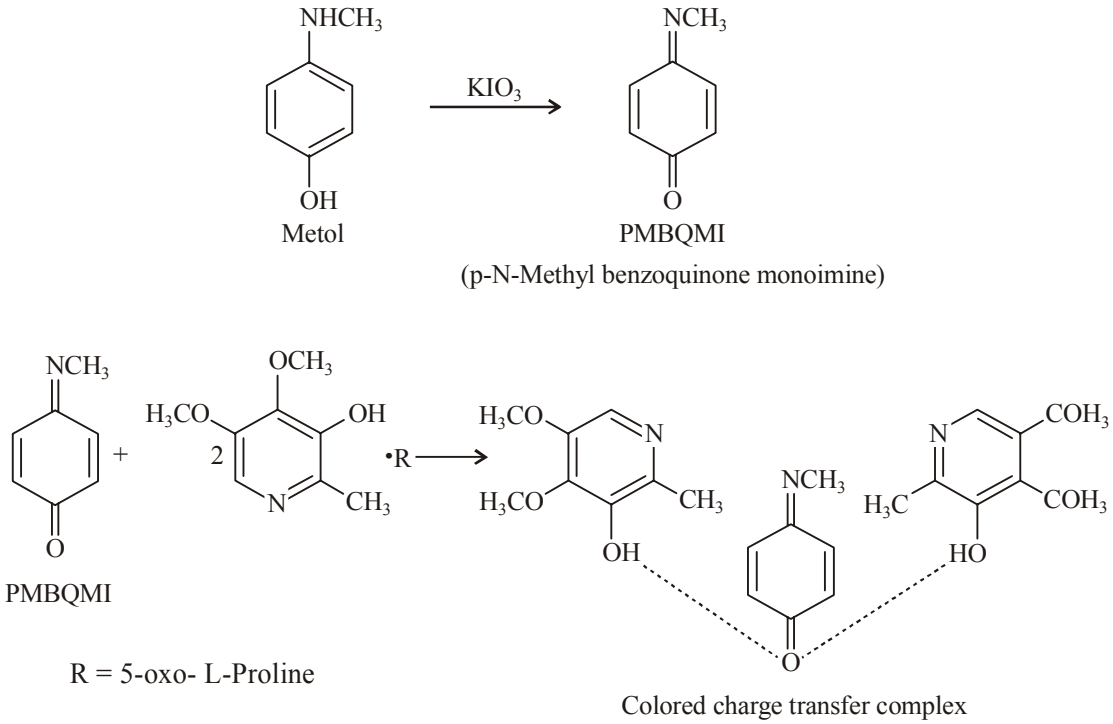
These methods are based on the oxidative coupling of MTD with MBTH in the presence of Fe (III) and Ce (IV), respectively. Under the reaction conditions, MBTH on oxidation with Fe (III) or Ce (IV) loses two electrons and one proton forming an electrophilic intermediate, which has been postulated to be the active coupling species. One mole of this intermediate reacts with MTD by an electrophilic attack on most electrophilic site of MTD to form colored species as shown in **Scheme 1**.



Scheme 1

Method C

The color development is due to the formation of a CT complex as represented in **Scheme 2**. The composition of colored species formed between PMBQMI and MTD can be explained as per the analogy of earlier workers.



Scheme 2

In order to ascertain the optimum wavelength of maximum absorption (λ_{max}), the spectra were scanned in the wavelength region of 400-800 nm against a corresponding reagent blank. The reagent blank absorption spectrum of each method was recorded against solvent employed in each method. The results were graphically presented in the Figs. 1-3.

The Beer's law plots of these systems are recorded graphically in Figs. 4-6. Beer's law limits, molar absorptivity, sandell's sensitivity and optimum photometric range (Figs.7-9) for MTD in each method developed with mentioned reagents were calculated. Least square regression analysis was carried out for getting the slope, intercept and correlation coefficient values. These were recorded in Table 1.

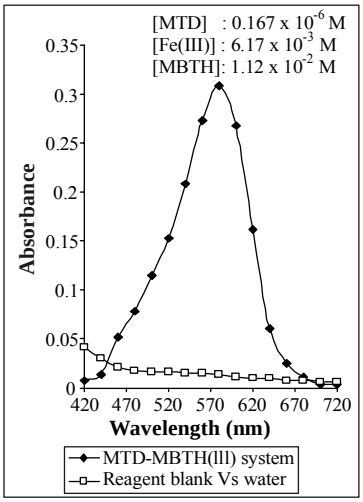


Fig. 1: Absorption spectra of MTD with MBTH-Fe (lll) system and its reagent blank

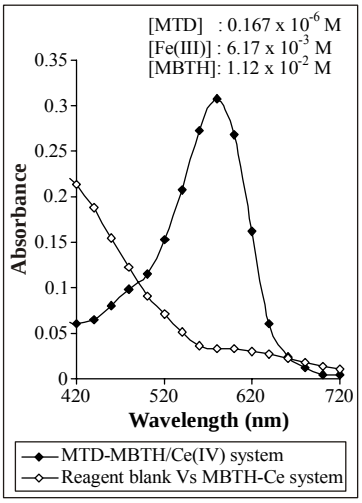


Fig. 2: Absorption spectra of MTD with MBTH-Ce (IV) system and its reagent blank

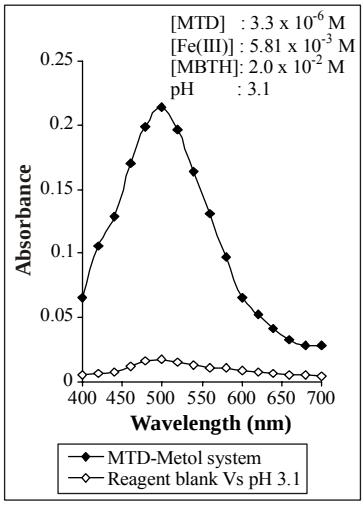


Fig. 3: Absorption spectra of MTD with metol system and its reagent blank

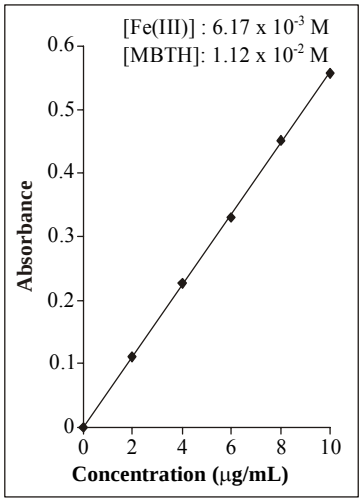


Fig. 4: Beer's law plot of MTD with MBTH-Fe (lll) system

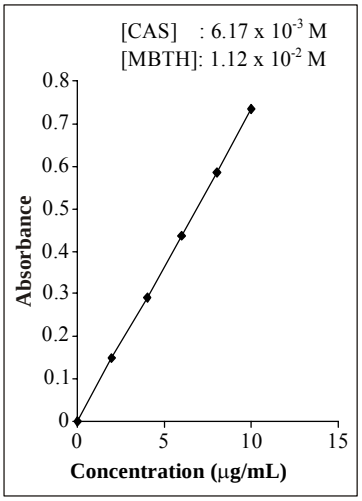


Fig. 5: Beer's law plot of MTD with MBTH-Ce (IV) system

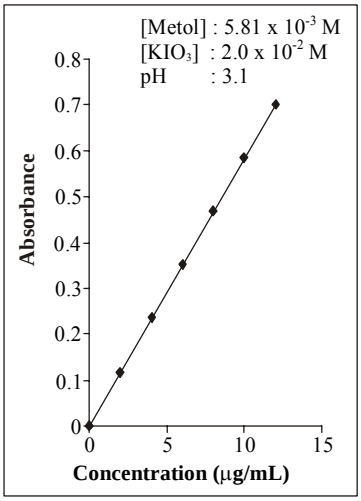


Fig. 6: Beer's law plot of MTD with metol system

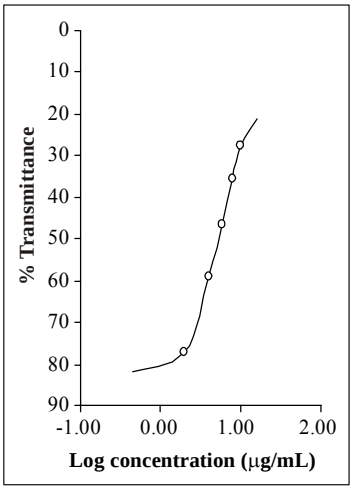


Fig. 7: Ringbom plot of MTD with MBTH-Fe (lll) system

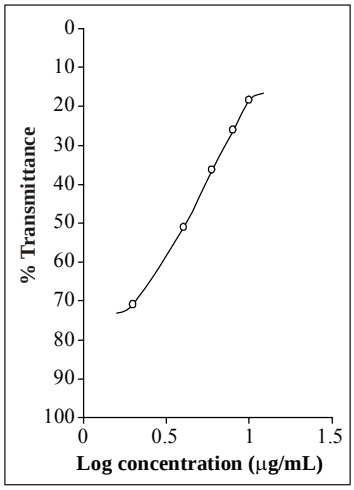


Fig. 8: Ringbom plot of MTD with MBTH-Ce (IV) system

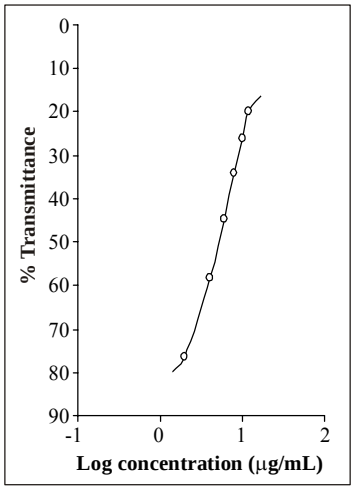


Fig. 9: Ringbom plot of MTD with metol system

Table 1: Optical characteristics and regression analysis parameters, precision and accuracy of the proposed methods for MTD

Parameter	Method A	Method B	Method C
λ_{max} (nm)	580	580	500
Beer's law limits (μg/mL)	2-10	2-10	2-12
Molar absorptivity (L. mole^{-1} cm^{-1})	1.70 x 10^2	2.17 x 10^3	1.755 x 10^3
Detection limits (μg/mL)	1.7533	0.1104	0.0602
Sandell's sensitivity (μg /cm ^{2}/0.001 absorbance unit)	0.0175	0.0136	0.0169
Optimum photometric range	110-690	8-24	12-48
Regression equation (Y = a+ bc):			
Slope (b)	0.0558	0.0245	0.0584
Standard deviation of slope (Sb)	4.33 x 10^{-4}	5.09 x 10^{-5}	7.65 x 10^{-6}
Intercept (a)	0.000714	0.00048	0.0005

Cont…

Parameter	Method A	Method B	Method C
Standard deviation of intercept (Sa)	2.62×10^{-4}	8.21×10^{-4}	5.52×10^{-4}
Standard error of estimation (S_e)	3.62×10^{-4}	1.13×10^{-3}	8.10×10^{-4}
Correlation coefficient (r)	0.9997	0.9998	0.9999
% Relative standard deviation*	0.226	0.390	0.854
% Range of error (Confidence limits)*			
0.05 level	1.0003	0.793	0.762
0.01 level	1.568	1.244	1.195
% Error in bulk samples**	0.44	– 0.19	– 0.59

* Average of six determinations ** Average of three determinations.

Interference studies were conducted to see the influence of excipients with the proposed methods. 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 2: Assay and recovery of in dosage forms

Method	Pharmaceutical formulation	Labelled amount (mg)	Proposed method			Found by reference method ± S.D	% Recovery by proposed methods** ± S.D
			Amount found* (mg) ± S.D	t (value)	F (Value)		
A	Tablet	500	504 ± 0.013	1.271	1.401	498 ± 0.009	99.88 ± 0.25
B	Tablet	500	499 ± 0.011	0.628	1.257	501 ± 0.012	100.12 ± 0.81
C	Tablet	500	498 ± 0.016	0.537	1.509	505 ± 0.011	100.83 ± 0.54

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.

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