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Research Article

ASSAY OF TRICLABENDAZOLE IN PHARMACEUTICAL FORMULATIONS BY VISIBLE SPECTROPHOTOMETRY

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ABSTRACT

Three simple and sensitive procedures (methods M_1, M_2 and M_3) for the assay of triclabendazole in pure form and formulations are described. Methods M_1, M_2 andM_3 are based on the selective redox - reactions of triclabendazole with an excess of oxidant(chloramine T) (CAT) in methodM_1 or NBS in method M_2 or in$AM-H_2SO_4$method M_3) in acidic medium. The unreacted oxidant is then estimated calourimetrically by using an oxidisable dye (galocynine GC) in method M_1 ((λ_{max} 540 nm) Celesta mine blue in method M_2 (λ_{max} 540 nm) and ammonium molybdate in method M_3 (λ_{max} 660 nm). The variable parameters in all these methods have been optimized. The results were statistically validated.

Keywords: Triclabendazole. CAT, NBS, CB.

INTRODUCTION

Triclabendazole (TB) is an antifungal drug. It is chemically known as 1H-Benzimidazole, 5- Chloro –6-(2, 3- dichlorophenoxy), –2-(methyl thio) and it is only available in Egypt as oral tablets fasinex (250 mg). A number of methods such as HPLC [1-11] and UV[1, 2] were reported for estimation of TB. The present paper describes three simple and sensitive spectrophotometric methods (M_1, M_2 andM_3) for the determination of TB based on its tendency to react with oxidizing agents such as CAT (It acts as a selective oxidising agent in both acid and alkaline media[14,15]). , NBS(It's more important application is an oxidant and as a reagent for a number of interesting substitution and addition[16, 17]reactions.)and ammonium molybdate. They act as selective oxidizing agents for the drug triclabendazole.

EXPERIMENTAL

Instruments

A Milton Roy spectronic 1201 and systronic 106 digital spectro- photometers were used for the spectral and absorbance measurements. An Elico Li-120 digital pH meter was used for pH measurements.

Reagents and solutions

All the reagents were of analytical grade and all the solutions were prepared in double distilled water. Freshly prepared solutions were always used. Aqueous solution of CAT solution (Loba: 0.02%, 7.10×10^{-4}M), GC solution (Chroma: 0.01%, 2.969×10^{-4}M) and HCl (E.Merck: 5.0 M) for methodM_1,N B S Solution(BDH: 0.088%, 4.94×10^{-3} M), AcOH solution (Qualigens: 5%; 8.75×10^{-1}M) for method M_2 and Ammonium molybdate solution (BDH: 2%: 5.1×10^{-2}M) and H_2SO_4 (BDH) for method M_3.

Standard drug solution

1 mg/ml stock solution of drug (TB) was prepared by dissolving 100 mg of drug was initially dissolved in 50 ml glacial acetic acid and made up to 100 ml with double distilled water. The working standard solutions of TB (20 μg/ml method M_1; 50μg /ml method M_2 and 80 μg/ml method M_3) were prepared by further diluting the stock solution with acetic acid.

Sample drug solution

To compare the results obtained by proposed methods, the author has developed methodin the laboratory according to literature methods for determination of TB in pure form as the tablets are not available in Indian market.

Portions of drug 50 mg was dissolved in 10 ml of isopropanol and shaken well and filtered and removed impurities if any. The filtrate was diluted in isopropanol to get 1 mg /ml. Stock solution was further diluted as in standard solution preparation.

Recommended procedures

Method M_1

Aliquots of standard drug (TB : 0.5-3.0 ml, 20 $\mu g.ml^{-1}$), 1.25 ml 5.0 M HCl and 2 ml of (7.1×10^{-4}M) CAT solutions were transferred into a series of 25 ml calibrated tubes and the volume in each tube was made up to 15.0 ml with distilled water. After 20 min. 10 ml of GC (2.96×10^{-4}M) was added and mixed thoroughly and the absorbances were measured after 10 min. at 540 nm against distilled water. Blank was prepared appropriately. The decrease in absorbance corresponding to consumed CAT, which in turn to the drug quantity was obtained by subtracting the absorbance of the blank solution from that of the test solution. The calibration graph was drawn by plotting the decrease in the absorbance of the dye (GC), against amount of drug. The amount of drug in any sample was computed from its calibration graph.

MethodM_2

To the aliquots of the standard drug (TB : 0.5-3.0ml, 50 μg.ml-1) solution taken in a series of 25ml calibrated tubes, 1.25 ml of 5.0 M HCl and 2.5 ml. of NBS (5.618×10^{-4} M) were added and the volume was made up to 15 ml with distilled water. After 10 min. 10 ml of CB (5.497×10^{-4}M) was added to each tube and mixed thoroughly. After 5 min. the absorbances were measured at 540 nm. against distilled water. The blank (omitting drug) and dye (omitting drug and oxidant) solutions were prepared in a similar manner and their absorbances corresponding to consumed NBS, and in turn to drug concentration, were obtained by subtracting the decrease in absorbance of test solution (dye minus test) from that of the blank solution (dye minus blank). The calibration graph was drawn by plotting the decrease in the absorbance of the dye (CB) against the amount of the drug. The drug concentration in the sample was readout from the calibration graph.

Method M_3

Aliquots of the standard drug solution (TB: 0.5-3.0 ml, 50 μg.ml-1) were transferred into a series of 10ml calibrated tubes. Then 1.0 ml of (5.1×10^{-2}M) ammonium molybdate and 4.0 ml of conc. H_2SO_4 were added to each tube and the contents were heated for 20 min. in a boiling water bath. After cooling, the volume was made up to10 ml with ethanol. The resulting absorbance of the green colour was measured at 660nm against similarly prepared reagent blank. The amount of drug was calculated from its calibration graph.

For Pharmaceutical Formulations

As the tablets (250 mg) of TB were available only in Egypt the author has prepared them in the laboratory according to literature methods[12, 13].Tablet powder equivalent to 100 mg of TB was extracted with 3×20 ml portions of glacial acetic acid and subsequently diluted to 100 ml with acetic acid for Methods M_1, M_2 andM_3.

RESULTS AND DISCUSSIONS

In the present investigations, the author proposes a sensitive indirect spectrophotometric procedure for the determination of TB. The principle involved in this procedure is quantitative decolourisation of gallocyanine (GC), by unreacted CAT. The probable sequences of reaction in two steps based on analogy are presented in scheme-1. In the present investigations, the author has proposed a simple, selective and sensitive spectrophotometric method for the assay of TB using NBS/CB. This method involves two steps. First step (I) is the oxidation of the drug (TB) with NBS. The second step IIb is the quantitative decolourisation of CB with the unreacted NBS.

TB + CAT → Products of oxidation + unreacted CAT

(Excess)

CAT + GC ⟶ Unreacted dye + mixture of compounds with

(Unreacted) (coloured) rupture of conjugated system

(Corresponds to reacted, colourless not stoichiometric as several alternate pathways possible)

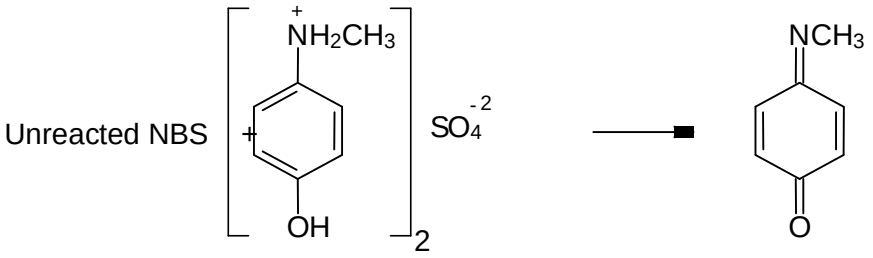
Scheme 1

Step I

TB + NBS ⟶ Oxidation product of TB + unreacted NBS

(Excess)

Step IIa



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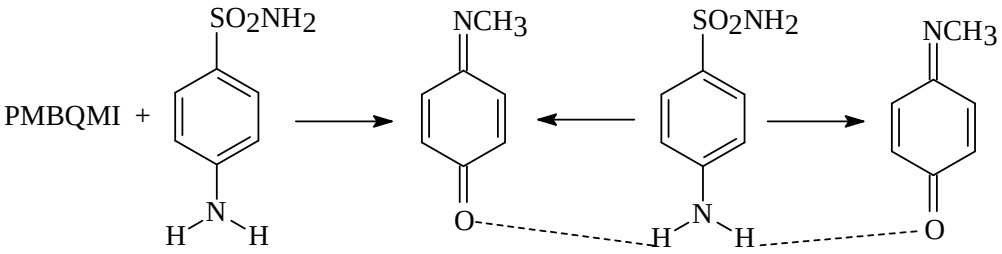

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Step IIb

Unreacted NBS+ CB ⟶ Oxidation products of dye + Unreacted CB

(Colourless through disruption (coloured)

ofchromophores and auxochromes)

Scheme 2

The chemistry of molybdenum is complicated. It forms compounds corresponding to oxidation numbers +2 to +6. The most stable and commonly encountered compounds of molybdenum are derived from MoO_3. The molybdenum compounds corresponding to the oxidation states ranging from +2 to +5 are mostly complex species.
Ammonium molybdate in conc. H_2SO_4 has been used for the determination of amitriptyline HCl[18]. In the present investigation the same reagent has been used for the determination of triclabendazole. The coloured species formation appears to be due to reduction of isopolyanionicmolybdate species to molybdenum blue.

Method M_1 (CAT/GC) for TB

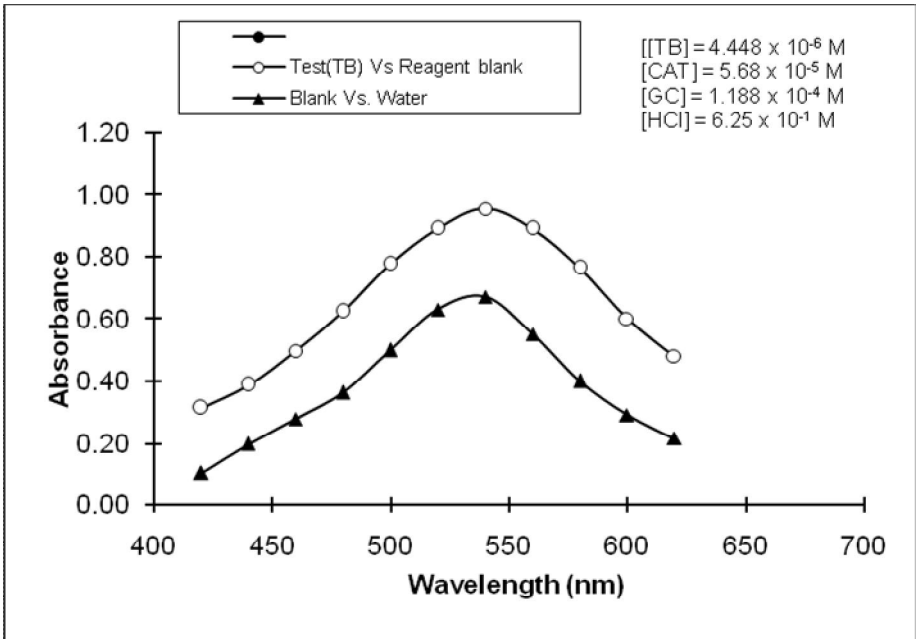
In order to ascertain the optimum wave length of maximum absorption (λ_{max}) of the final colour existing in the procedure specified amount of drug TB was taken and preceded as under procedure (M_1). The absorption spectra of the test solution versus distilled water, blank (omitting drug) versus distilled water and dye (GC) (omitting drug and CAT) versus distilled water were scanned on a spectrophotometer in the wave length region of 400-700nm. The absorption spectra of the test, blank and dye solution have shown the maximum absorbance at the same wave length shown and these results are graphically presented in Fig.1. Similarly in the nature of spectra indicated that the product formed, because of interaction of drug-CAT, CAT-dye does not affect the accuracy of the determination.

Method M_2 (NBS/CB) for TB

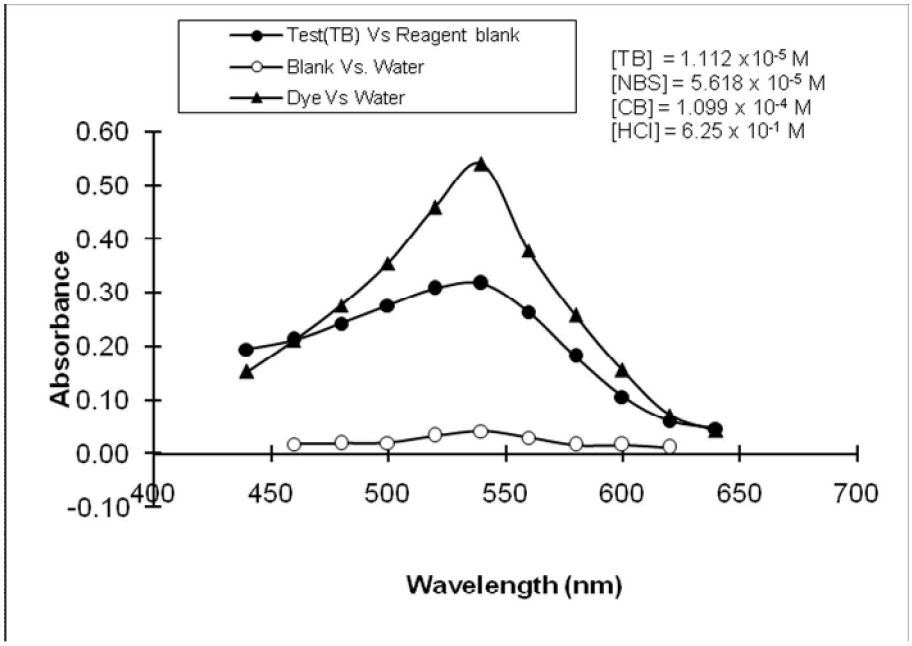
In order to ascertain the optimum wave length of maximum absorption (λmax) of the final colour obtained, a specified amount of the drug (TB) was taken, maintaining the other parameters as given in the recommended procedure as given in method (M_2). The absorption spectra of the test solution versus distilled water, blank (omitting drug) versus distilled water and dye (CB) versus distilled water were scanned on a spectrophotometer in the wave length region of 400-700nm The absorption spectra of the test, blank and dye solution have shown the maximum absorbance at the same wave length. The results are graphically presented in Fig.2.

Method M_3 (AM/H_2SO_4) for TB

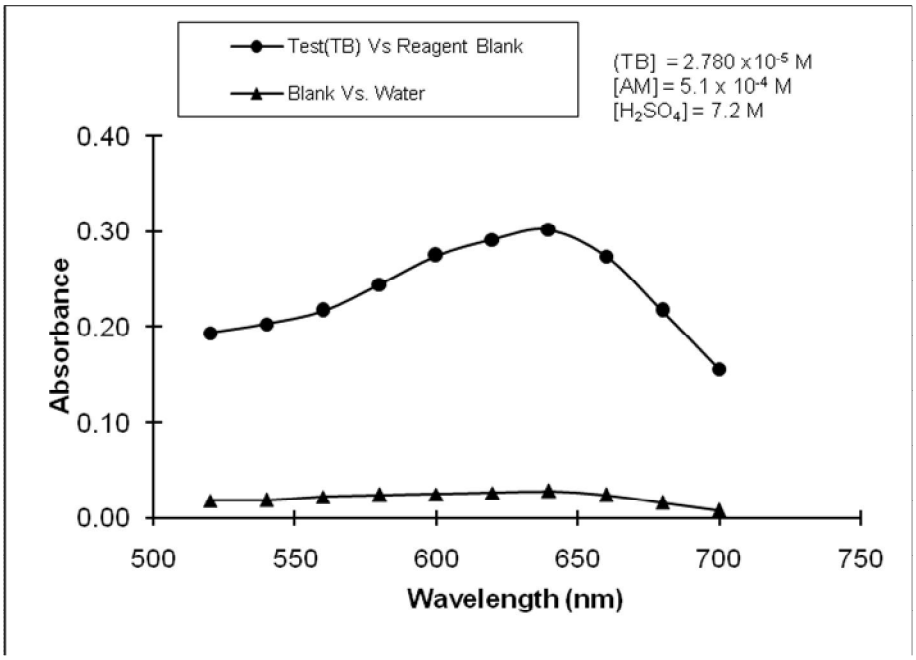
In order to ascertain the optimum wavelength of maximum absorption (λ_{max}) of the final colour existing in the procedure (M_3) specified amount of drug (TB) was taken and proceeded as under procedure (M_3). The absorption spectra of the test solution blank (omitting drug) was scanned on a spectrophotometer in the wave length the region of 400-800nm. These results are graphically presented in Fig. 3.The coloured species in each method shows characteristic absorption maximum at 660nm for both TB.



Absorption spectrum of TB-CAT/GC (M_1)



Absorption spectrum of TB-NBS/CB (M2)



Absorption spectrum of TB-AM/H2SO4 (M3)

The optimum conditions for the colour development of methods M_1, M_2 andM_3were established by varying the parameters one at time, keeping the others fixed and observing the effect produced on the absorbance of the coloured species. The colored solutions exhibited by λ_{max} at 540 nm, 540 nm and 660 nm respectively for method M_1, M_2 andM_3

Table 1: Optical and Regression Characteristics, PRECISION and Accuracy of the proposed methods FOR TB

λ_{max} (nm)	540	540	660
Beer's Law Limits ($\mu g.m1^{-1}$)	1-6.0	1-6.0	2.5-15
Detection limit ($\mu g.m1^{-1}$)	3.4×10^{-3}	3.259×10^{-4}	1.133×10^{-1}
Molar absorptivity ($mole^{-1}cm^{-1}$)	6.384×10^{4}	2.85×10^{4}	1.089×10^{4}
Sandell's sensitivity ($\mu g.cm-2$ / 0.01 absorbance unit)	5.617×10^{-3}	1.261×10^{-2}	3.3×10^{-2}
Regression equation (y=a+bc)			
Slope (b)	7.1×10^{-2}	7.917×10^{-2}	3.0002×10^{-2}
Standard deviation on slope (S_b)	2.0694×10^{-4}	2.006×10^{-4}	1.165×10^{-4}
Intercept (a)	5.333×10^{-4}	6.666×10^{-5}	8.0×10^{-4}
Standard deviation in intercepts (S_a)	8.059×10^{-4}	7.8122×10^{-4}	1.133×10^{-3}
Standard error of estimation (S_e)	8.764×10^{-4}	8.393×10^{-4}	1.219×10^{-3}
Correlation coefficient (r)	0.9999	0.9999	0.9999
Relative standard deviation (%)*	0.4113	0.3697	0.3393
% rang of error (confidence limits)*			
0.05 level	0.4318	0.3881	0.3562
0.01 level	0.6773	0.6109	0.5587
% Error in bulk samples**	0.2464	0.31545	0.09900

*Average of six determinations considered
** Average of three determinations

Table 2: Assay of TB in Pharmaceutical Formulations

	Labeled	**Amount found by proposed methods ****			**Reference**	**% Recovery by proposed methods*****		
Formulations*	amount	M_1	M_2	M_3	method	M_1	M_2	M_3
	mg	**CAT/GC**	**NBS/CB**	**AM/H_2SO_4**		**CAT/GC**	**NBS/CB**	**AM/H_2SO_4**
Tablets	250	248.43 ± 1.79	248.33 ± 1.30	249.91 ± 3.03	250.43 ± 1.98	99.37 ± 0.71	99.33 ± 0.52	99.96 ± 1.21
		F = 1.22	F = 2.32	F = 2.33				
		t = 0.90	t = 1.07	t = 0.62				
Tablets	250	249.70 ± 0.50	249.73 ± 0.45	249.72 ± 0.47	249.78 ± 0.38	99.88 ± 0.20	99.89 ± 0.18	99.88 ± 0.19
		F = 1.78	F = 1.42	F = 1.56				
		t = 0.99	t = 0.99	t = 0.99				
cream	250	248.34 ± 2.27	248.59 ± 1.61	249.54 ± 3.28	249.85 ± 2.43	99.32 ± 0.91	99.43 ± 0.64	99.81 ± 1.31
		F = 1.14	F = 2.28	F = 1.80				
		t = 0.61	t = 0.55	t = 0.57				
Tablets	250	249.31 ± 0.60	249.03 ± 1.06	249.35 ± 0.57	249.56 ± 0.76	99.72 ± 0.46	99.61 ± 0.42	99.74 ± 0.22
		F = 1.57	F = 1.94	F = 1.75				
		t = 0.50	t = 0.65	t = 0.43				

* Formulations from four different pharmaceutical companies.
** Average + standard deviation on six determinations, the t- and F – test values refer to comparison of the proposed method with the reference method.
Theoretical values at 95% confidence limit, F= 5.05, t = 2.57.
*** Recovery of 10 mg added to the pre-analyzed pharmaceutical formulations (average of three determinations).

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