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Determination of Triclabendazole by Visible Spectrophotometry

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Three simple, accurate rapid and sensitive reproducible visible spectrophotometric methods (**A-C**) have been developed for the estimation of triclabendazole in pure and pharmaceutical dosage forms. Methods **A** and **B** are based on the formation of chloroform soluble ion-association complex formation under specified experimental conditions are described. method **C** is based on the oxidative coupling under specified experimental conditions are described. Two acidic dyes, wool fast blue BL (WFBBL, λ_{max} 580 nm method **B**) tropaeolin OOO (TPOOO, λ_{max} 480 nm, method **A**) are utilized. Method **C** (λ_{max} 520 nm) is based on the reaction of drug with brucine and sodium metaperiodate under acidic conditions forming coloured bruciquinone derivatives. Results of the analysis for these methods were validated statistically by recovery studies.

Key Words: Extractive, Spectrophotometric, Determinations, Triclabendazole.

INTRODUCTION

Triclabendazole is an antifungal drug. It is chemically known as 1*H*-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 triclabendazole. The present paper describes three simple and sensitive spectrophotometric methods (**A, B** and **C**) for the determination of triclabendazole based on it's tendency to form chloroform extractable ion-association complexes with acidic dyes belonging to different chemical classes, namely, wool fast blue BL (WFB BL, method **A**), tropaeolin OOO (TPOOO, method **B**). Method **C** is based on the oxidative coupling of drug with brucine and sodium metaperiodate under acidic conditions forming coloured bruciquinone derivatives.

EXPERIMENTAL

A Milton Roy spectronic 1201 and systronic 106 digital spectrophotometers were used for the spectral and absorbance measurements, an Elico LI-120 digital pH meter was used for pH measurements.

All chemicals reagents used were of analytical grade and the solutions were prepared in triply distilled water. Solutions of WFBBL or TPOOO (0.2 %) glycine-HCl buffer (pH 1.5); HCl (0.1 M); were prepared in triply distilled water. Aqueous solutions of brucine (5.067×10^{-3} M) $NaIO_4$ (9.35×10^{-3} M) and H_2SO_4 (2.3 M) were prepared.

Preparation of standard drug solution: 1 mg/mL stock solution of triclabendazole was prepared by dissolving 100 mg of drug was initially dissolved in 50 mL glacial acetic acid and made up to 100 mL with triply distilled water. The working standard solutions of triclabendazole (10 µg/mL method-**A**; 25 µg/mL method **B** and 40 µg/mL method **C**) were prepared by further diluting the stock solution with acetic acid.

Sample drug solution: As the tablets of triclabendazole are not available in India, the authors have prepared them in the laboratory according to literature methods[12,13].

To compare the results obtained by proposed methods, a portion of triclabendazole 50 mg was dissolved in 10 mL of isopropanol and shake well and filtered and removed impurities if any. The filtrate was diluted in isopropanol to get 1 mg/mL.The stock solution was further diluted as in standard solution preparation.

Recommended procedures

Method A: Into a series of 100 mL separating funnels containing aliquots of drug (triclabendazole: 0.5-3.0 mL, 25 µg mL^{-1}) solutions, 6 mL of 0.1 M HCl and 2 mL of (5.709×10^{-3} M) TPOOO solutions were added successively. The total volume of aqueous phase in each separating funnel was adjusted to 15 mL with distilled water. To each separating funnel, 10 mL of chloroform was added and the contents were shaken for 2 min. The two phases were allowed to separate and the absorbance of separated chloroform layer was measured at 480 nm against a similarly prepared reagent blank. The amount of drug was calculated from the calibrated curve.

Method B: Into a series of 100 mL separating funnels containing aliquots of standard drug (triclabendazole: 0.5-3.0 mL, 10 µg mL^{-1}) solutions 6 mL of buffer solution (pH 1.5) and 2 mL of (3.26×10^{-3} M) WFBBL solutions were added successively. The total volume of aqueous phase in each separating funnel was adjusted to 15 mL with distilled water. To each separating funnel 10 mL of chloroform was added and the contents were shaken for 2 min. The two phases were allowed to separate and the absorbance of separated chloroform layer was measured at 580 nm against a reagent blank prepared under similar conditions. The amount of the drug was deduced from the calibration graph.

Method C: Aliquots of the standard drug solution (triclabendazole: 0.5-3.0 mL, 250 µg mL^{-1}) were transferred into a series of 10 mL calibrated tubes. 3 mL of (5.067×10^{-3} M) brucine solution, 1.5 mL of (9.35×10^{-3} M)

$NaIO_4$ and 2 mL of (2.3 M) H_2SO_4 were added to each tube and total volume was made up to 10 mL with distilled water. The tubes were thoroughly shaken and placed in boiling water bath for 15 min. The reaction mixture was cooled to room temperature and made up to 10 mL with distilled water. The absorbance of each solution was measured at 520 nm against a reagent blank. The amount of triclabendazole present in its sample was computed from the appropriate calibration graph.

RESULTS AND DISCUSSION

The optimum conditions for the colour development method were established by varying one parameter at a time each method, keeping the others fixed and observing the effect produced on the absorbance of the coloured species.

In order to establish the optimum pH range (for method **A**) or acid strength (for method **B**) the triclabendazole was allowed to react with the resulting dye in aqueous solution buffered between pH 1-6 (method **A**) or in dilute HCl ranging from 0.05 -1.5 M (method **B**) and (the complex formed was extracted in to chloroform for absorbance measurement. The results show that a quantitative extraction was produced between pH 1.4-1.8 (method **A**) or with an acid strength of 0.08-0.12 M HCl (method **B**). All subsequent studies were carried out at pH 1.5 (method **A**) or 0.1 M HCl (method **B**). The pH was adjusted using a glycine-HCl buffer solution (this buffer was chosen on account of its elevated complexing ability, which could be used in over coming interference). The volume of this buffer added (4-10 mL) had no effect in method **A**. A 5 mL of pH 1.5 (method **A**) or 6 mL portion of 0.1 M HCl solution (method **B**) was found to be optimum. The minimum shaking time was determined by varying the shaking time from 1-10 min, although 1 min was sufficient, prolonged shaking had no adverse effect on the extraction and 2 min was selected for this study. The ratio of 2:3 (method **A** and **B**) of organic to aqueous phases was required for efficient extraction of the coloured species and lower reagent blank reading. It was found that better reproducibility and a lower reagent blank were achieved if the dye was purified by extraction with chloroform initially. In method **C**, the optimum conditions were found to be 2.2-2.4 M H_2SO_4, 2.5-3.5 mL of 5.067×10^{-3} M brucine and 1.3-1.6 mL of 9.35×10^{-3} M $NaIO_4$. Other oxidants such as Fe(III) Ce(VI), (IV), V(V), IO_3^- and $S_2O_8^{2-}$ were tried instead of IO_4^- and found to be inferior. The optical characteristics such as Beer's law limits, molar absorbitivity, Sandell's sensitivity, correlation coefficient (r), regression equation percent RSD and percent range of error (95 % confidence limit) are listed in Table-1.

The optical characteristics such as Beers' law limits, molar absorptivity Sandell's sensitivity for each method are given in Table-1. The precision of

TABLE-1
OPTICAL AND REGRESSION CHARACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED METHODS FOR TRICLABENDAZOLE

Parameters	Method **A** TPOOO	Method **B** WFBBL	Method **C** Brucine- IO_4^-
λ_{max} (nm)	480	580	520
Beer's Law Limits (μg mL^{-1})	1.25-7.5	0.5-3.0	2-12
Detection limit (μg mL^{-1})	6.904×10^{-2}	1.344×10^{-2}	3.366×10^{-2}
Molar absorptivity (mol^{-1} cm^{-1})	1.629×10^{4}	4.2049×10^{4}	1.089×10^{4}
Sandell's sensitivity (μg cm^{-2}/0.01 absorbance unit)	2.21×10^{-2}	8.503×10^{-3}	3.33×10^{-2}
Optimum photometric rang (μg mL^{-1})			
Regression equation (y = a + bc)			
Slope (b)	4.569×10^{-2}	1.177×10^{-1}	3.024×10^{-2}
Standard deviation on slope (S_b)	2.160×10^{-4}	5.418×10^{-4}	4.35×10^{-5}
Intercept (a)	7.33×10^{-4}	-3.333×10^{-4}	-2.0×10^{-4}
Standard deviation in intercept (S_a)	1.051×10^{-3}	5.275×10^{-4}	3.389×10^{-2}
Standard error of estimation (S_e)	1.1296×10^{-3}	5.667×10^{-4}	5.855×10^{-4}
Correlation coefficient (r)	0.9999	0.9999	0.9999
Relative standard deviation (%)*	0.4576	0.6043	0.6775
% Range of error (confidence limits)*			
0.05 level	0.4804	0.6344	0.7113
0.01 level	0.7534	0.9950	1.215
%Error in bulk samples**	0.2654	0.4273	-0.1239

*Average of six determinations considered; **Average of three determinations.

TABLE-2
ASSAY OF TRICLABENDAZOLE IN PHARMACEUTICAL FORMULATIONS

Formulation* (labeled amount in mg)	Amount found by proposed methods**			Reference method	Recovery by proposed methods***		
	M_{1a} TPOOO	M_{1g} WFBBL	M_{23} Brucine- IO_4^-		M_{1a} TPOOO	M_{1g} WFBBL	M_{23} Brucine- IO_4^-
Tablets (250)	248.65 ± 2.99 F = 2.18 t = 0.61	249.77 ± 1.80 F = 1.20 t = 0.39	250.23 ± 3.41 F = 2.96 t = 0.15	250.43 ± 1.98	99.46 ± 1.17	99.91 ± 0.72	100.09 ± 1.36
Tablets (250)	248.85 ± 0.29 F = 1.67 t = 3.15	248.86 ± 0.30 F = 1.56 t = 2.98	249.19 ± 0.71 F = 3.55 t = 1.41	249.78 ± 0.38	99.54 ± 0.11	99.54 ± 0.12	99.67 ± 0.28
Tablets (250)	248.16 ± 2.28 F = 1.14 t = 0.62	248.56 ± 2.48 F = 1.04 t = 0.45	249.77 ± 3.81 F = 2.44 t = 0.09	249.85 ± 2.43	99.26 ± 0.91	99.42 ± 0.99	99.91 ± 1.52
Tablets (250)	246.82 ± 1.10 F = 2.11 t = 2.69	248.30 ± 1.40 F = 3.38 t = 2.91	247.00 ± 1.06 F = 1.97 t = 2.57	249.56 ± 0.76	98.73 ± 0.44	99.32 ± 0.56	98.80 ± 0.42

*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).

each method was found by measuring absorbances of six replicate samples containing known amounts of drug and the results are obtained are incorporated in Table-1. Regression analysis using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each method and is presented in Table-1. The results obtained by the proposed and reference methods (UV) for dosage forms were compared statistically by the t-and F-tests Table-2.

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