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Spectrophotometric Determination of Tinidazole Using Promethazine and Ethyl Vanillin Reagents in Pharmaceutical Preparations

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ABSTRACT

Two simple sensitive and reproducible spectrophotometric methods have been developed for the determination of tinidazole either in pure form or in their tablets. The proposed methods are based on the reduction of the nitro group to amino group of the drug. The reduction of tinidazole was carried out with zinc powder and zinc dust and concentrated HCl at 90 ±5 °C for 15 min in water. Method A is based on Schiff's basses reaction used ethyl vanillin reagent and measurement of the yellow coloured species (λ_{max}: 470 nm),whereas method B is based on oxidative coupling reaction used promethazine hydrochloride reagent and sodium hypochlorite oxidation agent in alkaline medium to form red colored measurable at 525 nm.The working conditions of both methods have been optimized. Regression analysis of Beer 's law plots showed good correlation in the concentration ranges 5-65 and 2-50 µg ml^{-1} for methods A and B, respectively. The apparent molar absorptivity and Sandell sensitivity values are calculated to be 3.214x10^{3}and 1.028x10^{4} l. mol^{-1} cm^{-1}, and 0.0769 and 0.0267 µg cm^{-2},with LOD 0.552 µg ml^{-1}and 0.285µg ml^{-1}, LOQ 1.840µg ml^{-1} and 0.942µg ml^{-1},respectively, for methods A and B. The methods were successfully applied to the determination of tinidazole in bulk drug and its formulations. Excipients used as additives in formulations did not interfere. Statistical treatment of the experimental results indicates that the accuracy and precision of the methods are analytically acceptable. The validity of the methods was evaluated by parallel determination by an established procedure, and by recovery studies.

Keywords: Spectrophotometric, Tinidazole , Promethazine hydrochloride, Ethyl Vanillin, Pharmaceutical.

INTRODUCTION

Tinidazole is chemically named as (1-(2-ethylsulfonylethyl)-2-methyl-5-nitroimidazole. It is a 5-nitroimidazole derivative used as an antiamoebic, antiprotozoal and antibacterial drug [1]. Owing to its bactericidal and antiprotozoal activity, this chemotherapeutic agent inhibits the growth of both anaerobic bacteria and certain anaerobic protozoa, such as *Trichomonasvaginalis*, *Entamoebahistolytica*and *Giardia lamblia*. It is also an important constituent of modern multidrug therapies for Helicobacter pylori eradication regimes used to control ulcers [2,3]. Tinidazole has been found to be equally or more effective than metronidazole in the treatment of trichomoniasis, giardiasis, and amebiasis [4].

A number of analytical methods for the quantitative determination of tinidazole in pharmaceutical preparations and biological fluids are known. Proposed methods have mainly used in high performance liquid chromatography [5-

11], high performance thin layer chromatography [12-15], gas liquid chromatography [16-20], packed column supercritical fluid chromatography [21,22] voltammetry [23,24] polarography [25],capillary electrophoresis[26] flow injection analysis [27], UV-spectrophotometry [28-31] and derivative UV-spectrophotometry [32-39]. Most of the spectrophotometric methods found in the literature for the determination of tinidazole in the visible region involve initial reduction by treatment with zinc powder and HCl [40-49] followed by the diazotization and coupling of the resulting amine.

In the present study, two different spectrophotometric methods for the quantitative estimation of tinidazole have been developed after converting it to its reduced form by using zinc powder and HCl, as well as the reaction of its reduced product with ethyl vanillin and promethazine hydrochloride was studied to establish the optimum reaction conditions, optical characteristics, precision and accuracy of the proposed methods. The methods are simple, rapid, sensitive and are successfully applied to determine the tinidazole in their pharmaceutical formulations

MATERIALS AND METHODS

Apparatus

All spectrophotometric measurements were performed on Shimadzu UV-visible recording spectrophotometer UV-160 using 1 cm matched quartz cell was used for recording spectra and absorbance.

Chemicals and Reagents:

All chemicals and reagent used were of analytical grades and double distilled water was used throughout. Ethyl vanillin, phenothiazine, hydrochloric acid, zinc dust and methanol were obtained from BDH Chemical Ltd, England. Sodium hypochlorite, sodium hydroxide were obtained from Fisher Scientific UK Ltd, England. Tinidazole internal standard material was obtained from state company for Shiba Pharmaceutical& Chemicals Mfg. Co. Ltd., Sana'a Yemen.

Promethazine hydrochloride ($5x10^{-3}$M) was prepared by dissolving 0.0966 g of promethazine hydrochloride reagent in distilled water and diluted to 100 ml in a volumetric flask. Ethyl vanillin of 4% (w/v) was prepared by dissolving 4g in a 100 ml volumetric flask containing water. Sodium Hypochlorite (0.1%)was prepared by dilution of 1.25 ml of 8% sodium hypochlorite to 100 ml by distilled water in a volumetric flask.

Sodium Hydroxide (0.1M) was prepared by dissolving of 0.4 g of sodium hydroxide in distilled water and diluted to 100 ml in a volumetric flask with the same solvent.

Reduction of Nitro group and Preparation of the Standard Solution:

A stock standard solution containing 1000 µgml^{-1} tinidazole was prepared as follows: 0.1g of tinidazole was accurately weighed into a beaker, dissolved in 40 ml of water and treated with 500 mg of zinc dust and 5 ml of concentrated HCl. The mixture was heated in a water bath at 90 ± 5 C^{o} for 15 min . It Was filtered using a Whattman filter paper 41 to remove the insoluble matter. The residue was washed with 10 ml portions of distaled water and diluted to the mark in a 100 ml calibrated flask. The working standard solution of 100 µg ml^{-1} was obtained by dilution of stock solution with distaled water.

General procedure

Method A:

In method A, fresh aliquots (0.5- 6.5 ml) of standard 100 µg ml^{-1} reduced tinidazole solution were accurately measured and transferred in to a series of 10 ml volumetric flasks by means of a micro burette. To each of the above aliquots, 2.0 ml of 4% (w/v) of ethyl vanillin solution, 1ml of HCl concentrated were added, and mixed thoroughly and then the solution was heated in a water bath at 60-70°C for 15 min, and cooled to room temp. After cooling, the volume was brought up to the mark with distal water, mixed well and the absorbance of each yellow colored species was measured after 10 min. at 470 nm against reagent blank. The amount of tinidazol present in the sample solution was computed from its calibration curve.

Method B:

In method B, fresh aliquots (0.2- 5.0 ml) of standard 100 µg ml^{-1} reduced tinidazole solution were accurately measured and transferred into a series of 10 ml volumetric flasks by means of a micro burette. To each 1ml of 5 x10^{-3}M of promethazine hydrochloride solution, 1.5 ml of 0.1% sodium hypochlorite and 2 ml of 0.1M sodium

hydroxide were added. The volumes were made up to the mark with distilled Water. The absorbance of read colored chromogen was measured at 525 nm against a reagent blank .

In either method, a calibration curve was prepared by plotting the absorbance as a function of concentration of drug solution. The concentration of the unknown was read from the respective calibration graph or calculated from the regression equation deduced from the Beer's law data.

Procedure for dosage forms:
Twenty tablets were weighed and finely powdered. An amount of powder equivalent to100 mg of tinidazole was extracted with two 40 ml portions of water. The solution of tinidazole was treated with 1 ml of concentrated Hydrochloric acid and 500 mg of zinc dust was added in portions while shaking. After standing for 1hour at room temperature, the solution was filtered using a Whatman filter paper No 41 filter paper to remove the insoluble matter. The residue was washed with 10 ml portions of water three times, and the total volume of the filtrate was made up to 100 ml with water. The final working standard solution of reduced tinidazol containing 100 µg $ml^{-1}$was prepared by further dilution. This reduced solution was suitably diluted and analysed by taking a convenient volume as described under general procedure.

RESULTS AND DISCUSSION

Method A is based on Schiff base, method B is based on oxidative coupling and the absorption spectra of the reaction products used in method A and B absorption spectra were taken against reagent blank in the range 400-800 nm. The maximum absorption wavelength for tinidazole was found to be 470 nm for method A and 525 nm for method B. The absorption spectrums of the colored product against reagent blank of both the methods are shown in Figures 1.and 2 respectively. Under the experimental conditions, each colorless reagent blank showed a negligible absorbance at the corresponding λ_{max}.

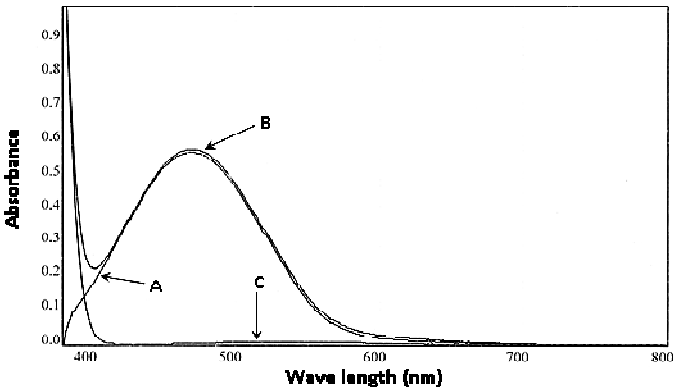


Figure 1. (A) Absorption spectra of 20 µg ml^{-1}tinidazole with ethyl vanillin against reagent blank. (B) complex against distilled water (C) blank against distilled water.

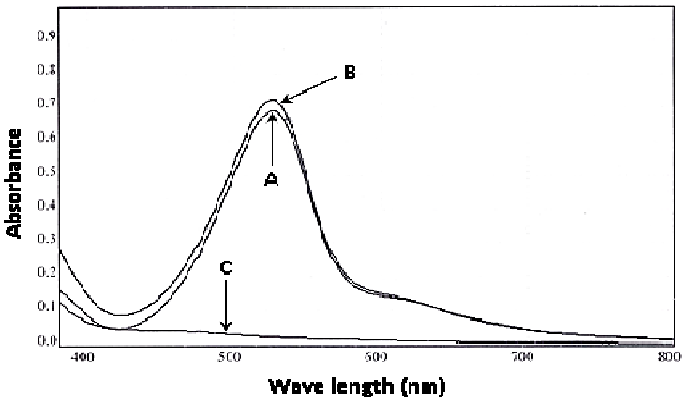


Figure 2. (A) Absorption spectra of 30 µg ml^{-1}tinidazole phenothiazine against reagent blank. (B) complex against distilled water (C) blank against distilled water.

Method development

Method A:

The optimum experimental conditions were established by varying one parameter at a time and observing its effect on the absorbance of the coloured species.

Effect of ethyl vanillin reagent:

When various concentrations of ethyl vanillin solution were added to fixed amount of the drug solution,2.0 ml of (4%) ethyl vanillin solution was found enough to develop the colour to its full intensity and give a minimum blank value and was considered to be optimum for concentration range of 5.0-65µg ml^{-1} of tinidazole.

Effect of Temperature:

The resulting product of the proposed method were studied at different temperatures. The results indicate that the absorbance values remain constant in the temperature range 20-80 C^o in a water bath for 15 min. It was observed that the obtained colored product were stable up to 70 C^o. However, no considerable improvements were occurred above 80 C^o therefore 60-70 C^o was selected as optimum temperature in this method.

Effect of Reaction Time:

The colour intensity reached its maximum after the drug tinidazole had been reacted immediately with ethyl vanillin solution and then the solution was heated in a water bath at 60-70C^o for 15 min, and cooled to room temp and stable after 5 min, therefore 10 minutes development time was selected as optimum in the general procedure. The colour obtained was stable for at least 24 hours.

Effect of order addition of reactants:

To obtain optimum results, the order of addition of reagents should be followed as given under the procedure, otherwise a loss in colour intensity was observed.

Method B:

The effect of various parameters on the absorption intensity of the dye formed was studied and the reaction conditions are optimized.

Effect of promethazine hydrochloride reagent:

The amount of promethazine hydrochloride reagent affected the intensity of the colored product, the absorbance of the colored product attained maximum intensity on using a volume of 1.0 ml of 5×10^{-3}M promethazine hydrochloride and was considered to be optimum for concentration range of 2.0-50 µg ml^{-1} of tinidazole.

Effect of Base:

It was found that the presence of a base led to increase the intensity of the produced product, therefore some bases such as NaOH, Na_2CO_3 and NH_4OH are examined and was found that all these bases gave almost equal intensity, so; NaOH was selected which was found that 2ml of this base give high sensitivity which selected in subsequent experiments.

Effect of Oxidant Concentration:

The product formation reached its maximum when 3-5 ml of 0.1 M of sodium hypochlorite solution were added to a mixture of tinidazole, promethazine hydrochloride and sodium hydroxide, therefore 1.5 ml was used in the procedure since it gives high sensitivity, minimum blank value and ensure a quantitative determination at the upper limit of the calibration graph.

Effect of Reaction Time:

The colour intensity reached its maximum after the drug tinidazole had been reacted immediately with promethazine hydrochloride in the presence of sodium hydroxide and became stable after 10 minutes, therefore 20 minutes development time was selected as optimum in the general procedure. The colour obtained was stable for at least 6 hours.

Effect of Temperature:

The resulting product of the proposed method were studied at different temperatures. The results indicate that the absorbance values remain constant in the temperature range 0-70 C^o, whereas, at higher temperatures the absorbance

value decreases, indicating the dissociation of the product on prolonged heating. The coloured product was stable for more than 6 hours at room temperature 25ºC. Therefore room temperature is selected in this method.

Effect of Order of Addition:

After fixing all other parameters, a few other experiments were performed to ascertain the influence of the order of addition of reactants. The order drug, sodium hypochlorite and sodium hydroxide followed by promethazine hydrochloride reagent after full colour development gave maximum absorbance and stability, and hence the same order was followed throughout the investigation.

Method validation :

Analytical appraisal:

Under the experimental conditions described, Beer's law, molar absorptivity and Sandell's sensitivities for tinidazol, are given in Table 1. Data of the regression analysis using the least squares method made for the calibration curves are also given in the same table. The limit of detection (LOD) was computed from the calibration graphs using the equation:

$$LOD \text{ or } LOQ = K \times SD/s$$

were K =3 for LOD and 10 for LOQ, SD is the standard deviation of three blank determinations and s is the slope of the calibration curve [50].This means that LOQ is approximately four times greater than LOD. LOD is well below the lower limit of the Beer's law range.

Table 1: Analytical parameters

Parameter	Method A	Method B
λ_{max} (nm)	470	525
Beer's law limits (µg ml^{-1})	5-65	2-50
Molar absorptivity (l mol cm^{-1})	$0.3214x10^4$	$1.028x10^4$
Sandell's sensitivity (µg cm^{-2})	0.0769	0.0267
LOD (µg ml^{-1})	0.552	0.285
LOQ (µg ml^{-1})	1.840	0.942
Regression equation (Y)*		
Slope (b)	0.0125	0.0361
Intercept (a)	0.0322	0.0134
Correlation coefficient (r)	0.9996	0.9984
Stability (hr.)	6	24
Color	Yellow	Red

** Y = a + b X where Y is the absorbance and X concentration in µg ml^{-1}.*

Accuracy and precision:

The accuracy and precision of the method were checked by analyzing five replicate samples within the Beer's law range containing the same amount of each drug. The lower values of RSD indicate the good precision and reproducibility of the method. The validity of the proposed procedure for the determination of tinidazolee in their pure state was checked by analyzing this drug using the proposed method. The results obtained for pure drug were reproducible with low relative standard deviations (RSD). The analytical results obtained from this study are summarized in Table 2. The accuracy of the methods is evident from the relative error (%) lying between 0.93 and 1.10 for method A and between 0.60 and 1.92 for method B. The relative standard deviation values which are less than 3 % for both methods indicate the high reproducibility of the methods. Further, the percentage range of error can be considered to be very satisfactory.

Interference:

The effect of the presence of some common excipients (glucose, lactose, starch, talc, sodium glycolate, sodium alginate, magnesium stearate, calcium gluconate, calcium carbonate, calcium dihydrogenorthophosphate and dextrose) on the selectivity of suggested method has been studied. The results indicate that there was no significant interference produced by these foreign substances (10-fold amount added) on the suggested methods.

Table 2: Accuracy and precision of the methods.

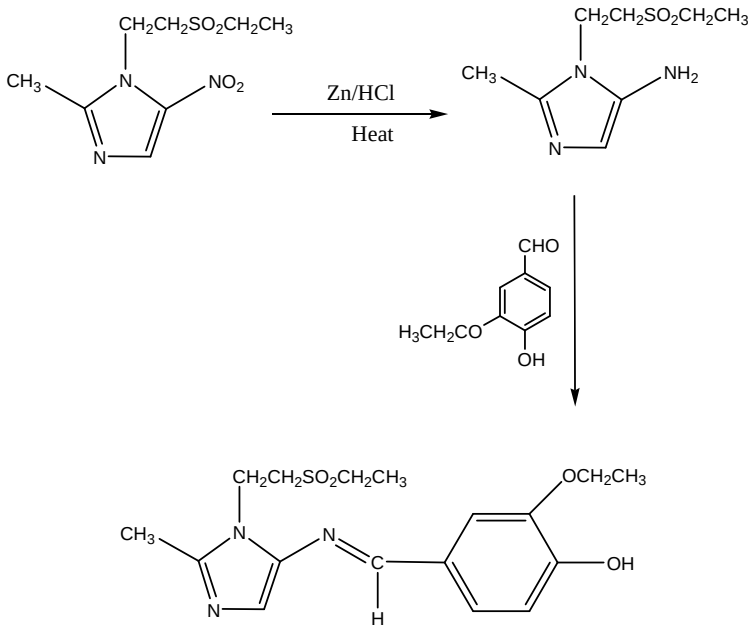
Method A					Method B				
Amount taken (µg ml^{-1})	Amount Found (µg ml^{-1})	Recovery%	Relative error %	RSD%	Amount taken (µg ml^{-1})	Amount Found (µg ml^{-1})	Recovery %	Relative error %	RSD%
10	9.89	98.90	1.10	2.71	10	10.12	101.20	+1.20	1.13
30	30.28	100.93	0.93	1.23	25	25.48	101.92	+1.92	0.70
50	49.52	99.04	0.96	2.35	40	39.76	99.40	-0.60	0.58

** Average value of five determination*
RSD–Relative standard deviation.

Composition of complex :

The composition of the formed complex had been established using Job's method [51]. The method was based on the measurement of series of solution in which molar concentration of tinidazole and ethyl vanillin (method A) vary but their sum remained constant, the other reagents added as mention in the general procedure and calibration graph .The results obtained show that 1:1 drug to reagent complex was formed at 470 nm. Tinidazole reacts with ethyl vanillin compounds according to the reaction shown in Scheme 1.

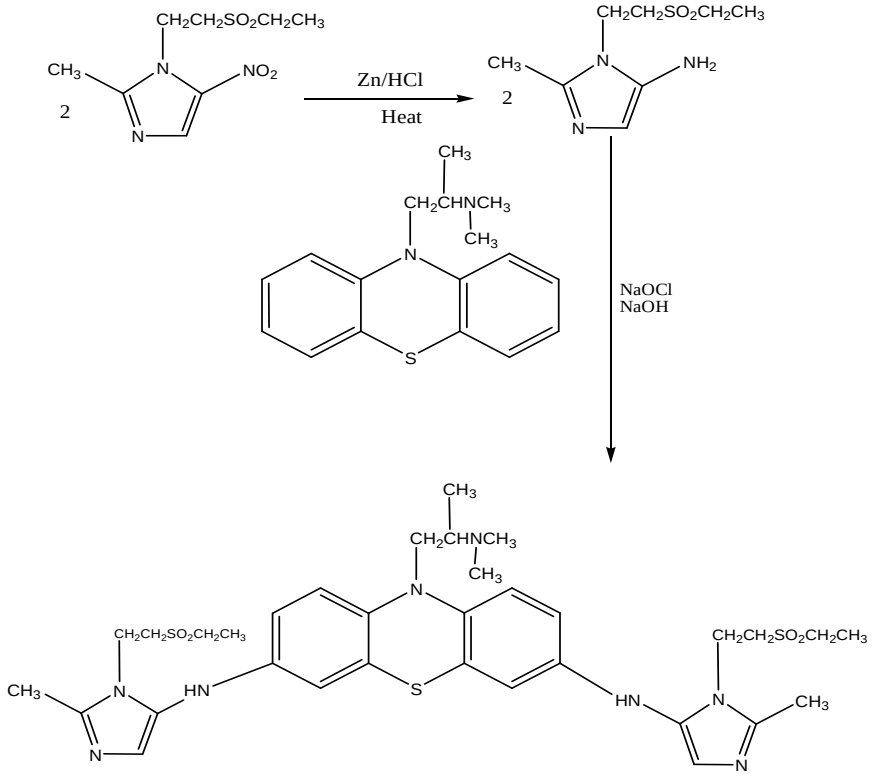
The stoichiometric ratio of tinidazole and promethazine hydrochloride ligand was investigated applying the continuous variation Job's methods using equimolar solutions of tinidazole and promethazine hydrochloride 1×10^{-3} M. It was found that drug forms a dye-coupled product with promethazine hydrochloride in the 1:2 ratio. The reaction may proceed as given in Scheme 2.



Scheme1: Stoichiometric ratio of tinidazol with ethyl vanillin.

Analytical Application:

The methods were applied to the determination of tinidazol in some selected representative tablets currently marketed in local stores. The results obtained were compared statistically by applying Students t-test for accuracy and F-test for precision with the official procedure [1] based on the measurement of the absorbance of the methanolic solution at 310 nm at 95 % confidence level with four degrees of freedom as shown in Table 3. The results showed that the calculated t-and F-values were less than the tabulated values indicating that there was no significant difference between the proposed and the comparison methods.



Scheme2: Stoichiometric ratio of tinidazol with promethazine hydrochloride.

Table 3: Results of assay of formulation containing tinidazol by the proposed methods.

Formulation and brand name***	Labelle amount mg tablet	Found* (% Recovery ± SD)		
		Method A	Method B	Official method
Tingyn[a] (Tablet)	500	101.43 ± 2.33 t= 0.52;F= 0.91	100.46 ± 0.729 t= 0.15;F= 0.04	102.25 ± 2.49
Enidazol[b] (tablet)	1000	101.12 ±1.47 t =1.86; F=2.28	98.86±2.12 t =2.16; F =3.20	102.15±1.85

** Average of five determinations*
*** Tabulated at t-value at 95 % confidence level 2.77*
**** Tabulated at F-value at 95 % confidence level 6.3*
Marketed by: a. Emessa Labs for Pharmaceutical Industries. Syria.
b. East India Pharmaceutical Works Ltd. India

Table 4: Assay of tinidazol drugs in some pharmaceutical formulations by standard addition method.

Pharmaceutical preparation	Drug content (mg)	Method A				Method B			
		Amount of drug in extract, µg	Amount of pure drug added, µg	Total found µg	Recovery of pure drug added, %	Amount of drug in extract, µg	Amount of pure drug added, µg	Total found µg	Recovery of pure drug added, %
Tingyn[a] Tablets	500	150	100	249.18	99.67	100	100	201.01	100.51
		150	200	349.43	99.84	100	200	299.57	99.86
Enidazol[b] Tablets	1000	200	100	301.52	100.51	150	100	249.93	99.97
		200	200	400.38	100.09	150	200	350.19	100.05

**Average of three determinations.*
[a]Emessa Labs for Pharmaceutical Industries – Syria.
[b]East India Pharmaceutical Works Ltd. India.

To ascertain the reliability of the methods, recovery test was performed by applying the technique of standard-addition. To a fixed amount of drug in the pre-analysed formulation, pure drug was added at two different levels and the total was found by the proposed methods. The test was repeated three times for each level. The results of

this study presented in Table 4 reveal that accuracy and precision of the methods were unaffected by the various co-formulated substances.

CONCLUSION

The proposed UV spectrophotometric methods are simple, cost effective and validated hence can be used for routine analysis of tinidazol in pharmaceutical Preparations.

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