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Method for Determination of Tinidazole using Direct UV-Visible Spectrophotometry and Differential Spectrophotometry in Pure and Tablet Dosage Forms

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Three simple, rapid, selective, precise and accurate spectrophotometric methods for the determination of tinidazole in tablet formulation were developed. The first method was based on the direct absorbance measurements of tinidazole in 0.5 N NaOH with a λmax of 368.6 nm and linearity range of 20 – 150 µg/ml. The second method was based on direct measurement of absorbance at 279.2 nm for tinidazole in 0.5 N HCl whereby the linearity range was 50-150 µg/ml. The third method was based on the differential spectra between tinidazole solution in 0.5 N NaOH and 0.5 N HCl. The maxima recorded was 368.8 nm while the minima was 276 nm with a linearity range of 20 – 120 µg/ml. The methods were validated by determining accuracy, precision, limit of detection, limit of quantitation and performing recovery studies. The developed methods were successfully applied in the analysis of commercial samples of tinidazole and could therefore be used in the routine analysis of tinidazole formulations.

Keywords: Tinidazole, direct colorimetry, UV-visible spectrophotometry, differential spectrophotometry

INTRODUCTION

Tinidazole (Figure 1) is a 5-nitroimidazole derivative used for the treatment of protozoal infections including giardiasis, amoebiasis and vaginal trichomoniasis [1,2]. The IUPAC name for tinidazole is 1-(2 ethylsulfonylethyl)-2-methyl-5-nitroimidazole [3].

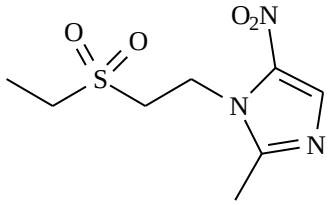

Figure 1. Chemical structure of tinidazole.

The British Pharmacopoeia (2009) specifies non-aqueous titration method using acetous perchloric acid for the assay of tinidazole [4]. Gas liquid chromatography, high performance liquid chromatography [5], voltammetry [6] and spectrophotometry [7-15] are some of the methods found in the literature for the determination of tinidazole. All these methods suffer the drawbacks of low sensitivity and involve tedious procedures such as extraction and use of costly reagents. The present study was aimed at developing methods overcoming these shortcomings yet simple, rapid and accurate.

EXPERIMENTAL

Instrumentation

A Shimadzu UV 1700 UV/Vis double beam spectrophotometer (Kyoto, Japan) with spectral band width of 1 nm, equipped with a 1.0 cm matched quartz cells, was used for spectrophotometric measurements.

Reagents and materials

All the chemicals and reagents used were of analytical grade. Sodium hydroxide pellets and hydrochloric acid (Loba Chemie Pvt., Mumbai, India) were procured while distilled water used for the dissolution of reagents was prepared in the laboratory. Tinidazole tablets (Amebamagma®, Wyeth, 300 mg) was procured from a local pharmacy. Tinidazole working

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standard was provided by Jaipur Pharmaceutical Work (Jaipur, India).

Stock solutions of tinidazole

Tinidazole powder (100 mg) was transferred into a 100 ml volumetric flask and dissolved in distilled water with ultrasonication for 15 min to give a stock solution with a concentration of 1000 µg/ml. Stock solutions were freshly prepared.

Calibration curves of tinidazole

Method I: The tinidazole stock solution was diluted in distilled water serially to a concentration range of 20-150 µg/ml tinidazole. A calibration curve of concentration versus measured absorbance was plotted. The spectral characteristics of tinidazole are given in Table 1.

Method II: Serially diluted aliquots of tinidazole stock solution were transferred and diluted with 0.5 N HCl to give a concentration range of 20-150 µg/ml of tinidazole. A calibration curve of concentration versus measured absorbance was plotted. The spectral characteristics of tinidazole are given in Table 1.

Method III: Serial dilutions of the tinidazole stock solution were made in 0.5 N HCl and in 0.5 N NaOH to give a concentration range of 20 - 150 µg/ml both for reference and test solutions, respectively. Difference spectra were obtained for tinidazole solutions in 0.5 N HCl in reference cell and that of tinidazole in 0.5 N NaOH in sample cell. The difference in absorbance between 368.8 nm and 276 nm was calculated to yield the amplitude. The differential spectral characteristics of tinidazole are given in Table 1 and Figure 2.

Sample analysis of the tablet formulation

Twenty Amebamagma®-300 mg tablets were weighed and powder equivalent to 100 mg of tinidazole was transferred to a 100 ml volumetric flask followed by ultrasonication for 15 min. The resultant solution was filtered through Whatman filter paper no. 41 into a second 100 ml volumetric flask. The filter paper was washed several times with distilled water. The washings were added to the filtrate and final volume was made up to 100 ml with distilled water.

Method I: 0.8 ml of filtrate of the sample solution was diluted to 10 ml with 0.5 N NaOH. These were treated as per the procedure for preparation of a calibration curve and amount of the drug present in sample computed from respective calibration curve.

Method II: 0.8 ml of filtrate of the sample solution was diluted to 10 ml with 0.5 N HCl. These were treated as per the procedure for preparation of calibration curve and amount of the drug present in sample computed from respective calibration curve.

Method III: 0.8 ml of filtrate of the sample solution was diluted to 10 ml with 0.5 N HCl and 0.8 ml of filtrate of the sample solution was diluted to 10 ml with 0.5 N NaOH. These were treated as per the procedure for preparation of calibration curve and amount of the drug present in sample computed from respective calibration curve.

The analysis of the tablet formulation was done in sextuplicate and results reported in Table 2.

RESULTS AND DISCUSSION

Method development

Three methods were developed for the analysis of tinidazole tablet dosage form using UV spectrophotometery. The first method was based on colorimetric determination of tinidazole in 0.5 N NaOH at the λmax of 368.6 nm. The second method was based on estimation of tinidazole in 0.5 N HCl at the λmax of 279.2 nm. The third differential spectroscopic method was based on difference in absorbance. By placing tinidazole dilution in 0.5 N NaOH in sample cell and tinidazole dilution in 0.5 N HCl in reference cell, the differential absorption spectra was recorded.

Table 1: Spectral characteristics and linearity data

Parameters	Method I	Method II	Method III
λmax	368.6 nm	279.2 nm	Maxima: 368.8 nm Minima: 276 nm
Beer's law limit (µg/ml)	20-150 µg/ml	50-150 µg/ml	20-120 µg/ml
Regression equation (y = a + bx)	y = 0.016 + 0.009x	y = 0.011 + 0.006x	y = -0.069 + 0.014x
Slope (δ)	0.009	0.006	0.014
Intercept (a)	0.016	0.011	-0.069
Coefficient of determination (r^2)	0.999	0.999	0.999
LOD	5.98 µg/ml	15 µg/ml	5.68 µg/ml
LOQ	19.93 µg/ml	50.08 µg/ml	18.95 µg/ml

LOD: Limit of detection; LOQ: Limit of quantitation.

Table 2: Recovery studies

	Amount of drug in preanalyzed sample (µg/ml)	Amount added (µg/ml)	Recovered Amount (µg/ml)	Accuracy (percentage recovery) (% Bias)	Precision		
					S.D.	% R.S.D.	±SE
Method I	79.88	5	5.03	100.60			
	79.88	10	9.97	99.70			
	79.88	15	14.99	99.93			
	79.88	20	20.01	100.05	0.3005	0.3002	0.09
	79.88	25	25.05	100.20			
	79.88	30	30.03	100.10			
	Mean			100.09			
Method II	79.88	5	5.01	100.20			
	79.88	10	9.97	99.70			
	79.88	15	15.04	100.26			
	79.88	20	20.01	100.05	0.2207	0.2206	0.04
	79.88	25	24.96	99.84			
	79.88	30	30.05	100.16			
	Mean			100.03			
Method III	79.88	5	5.09	101.8			
	79.88	10	10.04	100.4			
	79.88	15	14.99	99.93			
	79.88	20	20.00	100.00	0.720	0.7203	0.51
	79.88	25	25.06	100.24			
	79.88	30	29.97	99.90			
	Mean			100.37			

n = 6; S.D. = standard deviation; %R.S.D. = percentage standard deviation; %Bias = found-added/added × 100; SE = standard error.

The difference between absorption spectra maxima (368.8 nm) and minima (276 nm) were determined (Figure 2). The calibration curve was prepared by plotting the concentration against the difference in absorbance (ΔA) between the maxima and the minima.

Method validation

Linearity range

Calibration curves were plotted for each spectrophotometric method. The statistical parameters and regression equations were calculated from the calibration curves along with the standard error of the slope and the intercept as shown in Table 1. Regression analysis indicated a linear relationship between absorbance and concentration, within the ranges of 20 - 150 µg/ml, 50 – 150 µg/ml and 20 – 120 µg/ml for method I, II and III, respectively (Table 1).

Sensitivity

The International Conference on Harmonization (ICH) [16] guidelines on determination of limit of detection (LOD) and limit of quantitation (LOQ) define LOD as 3 s/δ while LOQ as 10 s/δ, where s is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte and δ is the sensitivity, the slope of the calibration curve. The LOD and LOQ values for methods I, II, III are shown in Table 1.

Selectivity/specificity

The comparison of standard spectra and spectra from tablet samples showed that the wavelengths of maximum absorbance and maxima/minima did not change. This showed that excipients did not interfere with quantitation of tinidazole in tablet by developed methods.

Accuracy

In this study, accuracy was determined by analyzing the recoveries of known amount of tinidazole added into preanalyzed sample of tinidazole tablets. To determine the precision of the methods, sextuplicate analyses were performed using each method. The percent recoveries were found to be 100.09 ± 0.09%, 100.03 ± 0.04% and 100.00 ± 0.51% for methods I, II and III, respectively (Table 2).

Precision

The interday precision (n=6) and accuracy study was carried out for each method and the amount of tinidazole recovered were 99.86 ± 0.0083, 100.06 ± 0.0013 and 100.13 ± 0.0059 for method I, II, III respectively (Table 3). The repeatability (n=6) was carried out for each method whereby the RSD was < 1 for all the methods.

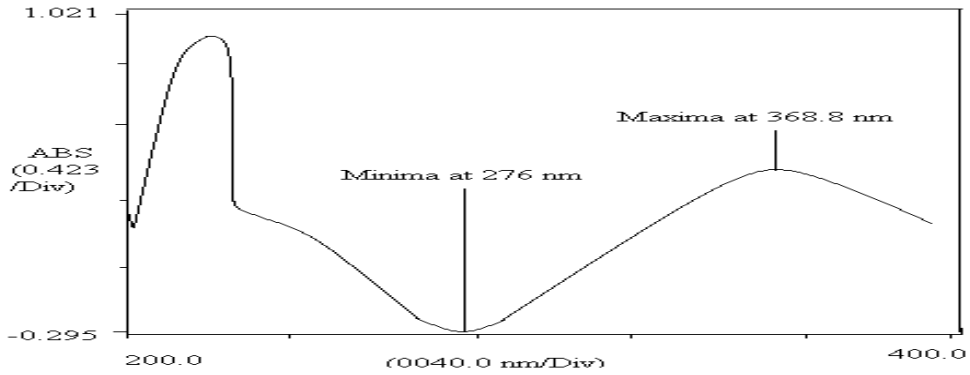

Figure 2. Difference UV-Visible absorption spectra of tinidazole.

Table 3: Interday and intraday precision and accuracy

			Method I	Method II	Method III
Interday	Added (µg/ml)		15	15	15
	Mean found (µg/ml)		14.98	15.01	15.02
	Precision	S.D.	0.0916	0.0361	0.0771
		%R.S.D.	0.6114	0.2405	0.5133
	Accuracy (Percentage recovery, %Bias)		99.86	100.06	100.13
	±S.E.		0.0083	0.0013	0.0059
Intraday	Added (µg/ml)		15	15	15
	Mean found (µg/ml)		15.01	15.02	15.05
	Precision	S.D.	0.0613	0.0201	0.0151
		%R.S.D.	0.4083	0.1338	0.1003
	Accuracy (Percentage recovery, %Bias)		100.06	100.13	100.33
	±S.E.		0.0037	0.0004	0.0002

n = 6; S.D. = standard deviation; %R.S.D. = percentage standard deviation; %Bias = found-added/added × 100; S.E. = standard error.

CONCLUSION

The assay results were 99.82 ± 0.1261 (n=6), 99.86 ± 0.0993 (n=6) and 99.80 ±0.1356 (n=6) for methods I, II and III, respectively.

In this study, direct UV spectrophotometric and differential spectrophotometric methods were developed and validated according to ICH guidelines. It was found that tinidazole can be directly determined in tablets in presence of excipients by using the developed spectrophotometric methods.

The methods developed were found to be simple, sensitive, accurate, precise and inexpensive with good recovery of the analyte. The developed methods can be applied for routine and analysis of tinidazole tablets.

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