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Development and validation of stability indicating UV spectrophotometric method for quantification of tinidazole

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Abstract
Simple, accurate, precise and economical UV spectroscopic method for estimation of tinidazole has been developed and validated. The wavelength of maximum absorbance selected is 317nm, which is the λmax value of tinidazole in phosphate buffer (pH = 6.8). Tinidazole shows linearity at the selected wavelength and obeys Beer's law in the concentration range of 3.0-30µg/mL with correlation coefficient of 0.9997. Recovery studies for tinidazole were performed and the percentage recovery was obtained in the range of 98.775-100.718% confirming the accuracy of the proposed method. The methods showed good reproducibility and recovery with % RSD less than 2. Statistical validation of the data obtained shows that the proposed methods can be used as stability indicating method which can be successfully applied for the routine analysis of drugs in commercial tablets.

Keywords: tinidazole, spectrophotometry, validation, stability indicating

Introductions

Tinidazole, chemical name is1-(2-ethylsulfonylethyl)-2-methyl-5-nitro-imidazole and chemical structure figure 1 [1, 2]. Its molecular weight is 247.273 g/mole [3]. It's one of the 5−nitroimidazole drugs, is antiprotozoal and antibacterial agent. The nitro-group of tinidazole is reduced by cell extracts of trichomonas. The free nitro-radical generated as a result of this reduction may be responsible for antiprotozoal activity. Chemically reduced Tinidazole was shown to release nitrites and cause damage to purified bacterial DNA in vitro [4] or inhibit DNA synthesis in microorganism [5]. The 5-nitroimidazole is an important class of imidazole-based drugs [6]. The 5-nitroimidazoles are a well-established group of protozoal and bactericidal agents [7].

The importance of imidazole can be realized by the fact that many drugs in use today contain this moiety and several nitroimidazole derivatives such as metronidazole, tinidazole, ornidazole, secnidazole and ronidazole have been used for the treatment of serious infections caused by anaerobic bacteria and protozoa [8].

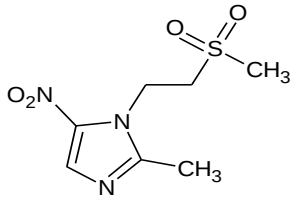

Fig 1: Chemical structure of tinidazole

Tinidazole is the subject of monograph in each of the BP and the USP. Either pharmacopeia recommends non-aqueous titration for determination of Tinidazole [9].

Validation of analytical procedure as defined by international conference on harmonization [10], is to demonstrate that it is suitable for its intended purpose. According to USP 2008 [11], Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for the intended analytical applications.

2. Materials and methods

2.1 Materials

All chemicals and organic solvents were of analytical grade. Distilled water was used in all experiments. Chemicals (suppliers) were as follows:

The standard of tinidazile (99.87%) was obtained from Azal Pharma. Tablet formulation Tinazol (General Medicines Company, Sudan) and Tinirem (Remedica Ltd.–Cyprus, Europe) were purchased from a local market with labeled amount 500 mg tinidazol. Methanol and Potassium dihydrogen monobasic (SDFD Ltd).

2.2 Instruments

Double beam UV 1800 ultraviolet visible spectrophotometer provided with matched 1-cm quartz cells (SHIMATZU-Japan) with temperature maintained at 25°C. pH Conductivity meter, Sartorius PP-20, USA was used for pH measurements. Horizontal Flow Oven WOF-155, DAIHAN Scientific Co. Ltd., Korea.

2.3 Methods

2.3.1 Preparation of reagents and solutions

2.3.1.1 Standard stock solution of Tinidazole

An accurately weighed amount 15.0 mg of tinidazole was dissolved in 50 mL of methanol to prepare a stock solution with concentration 0. 30 mg/mL.

2.3.1.2 Working standard

1.0 ml of standard solution was transferred to 20 mL volumetric flask, completed to the mark with buffer solution which prepared as bellow, final concentration obtained was 0.015mg/mL.

2.3.1.3 Buffer solution
Buffer solution of pH = 6.8 was prepared by weighing 2.73 g of potassium dihydrogen monobasic phosphate, dissolved in water, transferred to 1.0 L volumetric flask and completed to the mark with water, this solution adjusted pH = 6.8 by adding drops of 0.2 M NaOH which prepared by weighing 4.0 g, dissolved by water and completed to the mark (500 mL volumetric flask).

2.3.1.4 Blank solution
1.0 mL of methanol transferred to 20 mL volumetric flask, completed to the mark by buffer solution (diluent).

2.3.2 Stress degradation study
Four solutions of the drug were prepared in 0.1M sodium hydroxide, 0.1M hydrochloric acid and 30% hydrogen peroxide (taking 5 mL) for each one, the fourth sample was heated to 80°C to examine thermal degradation, cooled to room temperature, then samples treated as working standard, full scanned in wavelength region of 200-400 nm.

2.3.3 Determination of λ_{max}
Solution of tinidazole with concentration (0.015 mg/mL) was scanned in the wavelength region of 200-400 nm to determine the maximum absorbance.

2.3.4 Determination of optimum pH
The absorbance of series of Tinidazole working solutions have the same concentrations containing buffer varying the pH from 2.0 to 8.0 was measured at 317 nm.

2.3.5 Validation procedure

2.3.5.1 Selectivity
The spectrum of tinidazole (standard) was compared with that of sample which prepared by adding 10.3 mg of placebo (content of drug except tinidazole) which is equivalent to standard weight, then the sample treated as tinidazole working standard.

2.3.5.2 Linearity and range
To demonstrate the linearity for tinidazole, different aliquots, 0.2, 0.4, 0.6, 0.8, 1.0 to 2.0 mL of tinidazole stock solution were taken in a series of 20 mL volumetric flasks and diluted up to the mark with buffer to get required concentrations range of 0.003mg/mL to 0.03 mg/mL.

2.3.5.3 Accuracy
The study of accuracy was carried out at different concentrations of tinidazole 50, 100 and 150% each concentration is in three replicates.

2.3.5.4 Precision
The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day) in triplicate. Repeatability refers to use of the analytical procedure over a short period of time that was evaluated by assaying the samples the same day. Intermediate precision was assessed by comparing the assays on different days (2 days) and by different analyst, relative standard deviation calculated.

2.3.5.5 Robustness
The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions included pH (±0.1) and maximum absorbance (±2nm).

2.6 Applications of method to dosage form
The development and validated UV method was applied for determination tinidazole dosage form. Tinidazol tablet of 500 mg strength was evaluated. 10 tablets were powdered and powder equivalent to 500mg of drug was weighed. The weighed sample was dissolved in methanol (50 mL), the suspension was sonicated for 10 min, filtered, and 1.0 mL was taken in 20 ml volumetric flask to obtain 0.015 mg/mL final concentration (completed to the mark with buffer). Absorbance of the sample solution was recorded at determined λ_{max}, similarly, the assay of Tinirem tablets containing 500mg of tinidazole was carried out.

3. Results and discussion
3.1 Method optimization
The objective of the present work is to develop a UV method for determination of tinidazole and to validate the method by using various parameters.
The absorption spectra of tinidazole against reagent blank was taken in the range 200-400 nm. The maximum absorption wavelength for Tinidazole was found to be 317 nm. The absorption spectrum is shown in Figure 2.

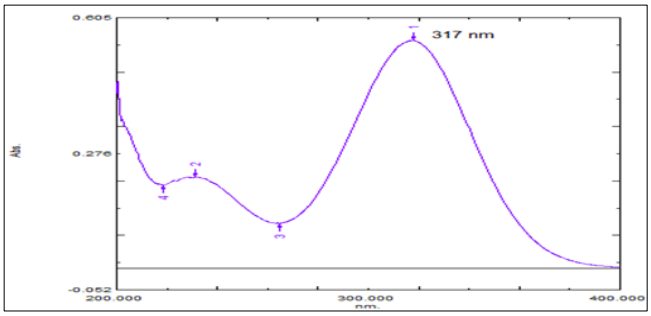

Fig 2: Spectrum of Tinidazole showing λmax at 317nm.

Forced degradation study shows that, tinidazole is degraded clearly in basic media, as shown in Figure 3.

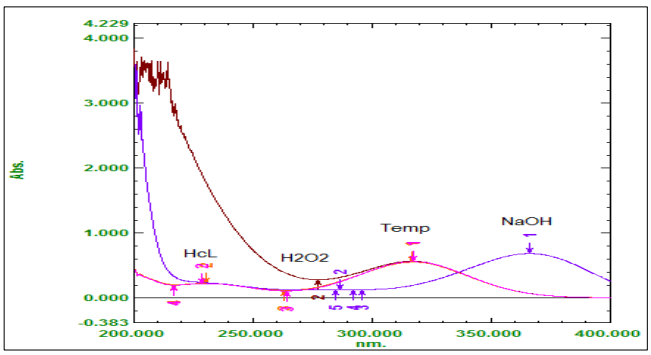

Fig 3: Under stress testing

The optimum pH was found to be 6.8. The Results revealed that the absorbance increased proportional to pH increase till 6.8, then it decreased obviously as shown in figure 4.

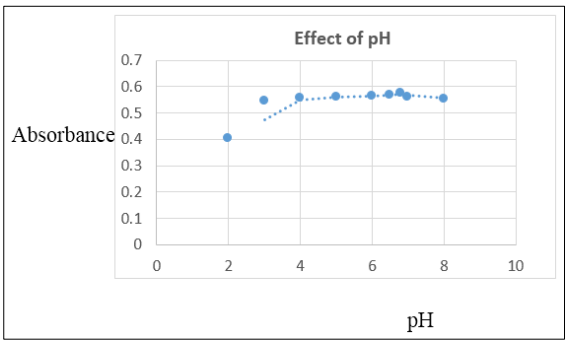

Fig 4: Effect of pH on absorbance

3.2 Method validation

The proposed method was validated according to international conference harmonization guideline for parameters: Selectivity, linearity and range, precision, accuracy, robustness, limit of detection (LOD) and limit of quantitation (LOQ).

3.2.1 Selectivity

The selectivity studies revealed absence of any excipient or impurity interference, since there is no absorbance peaks appeared at the same of λ_{max} of tinidazole as shown in figure 5. Maximum absorbance for standard and sample is 317 and 316.9 nm respectively.

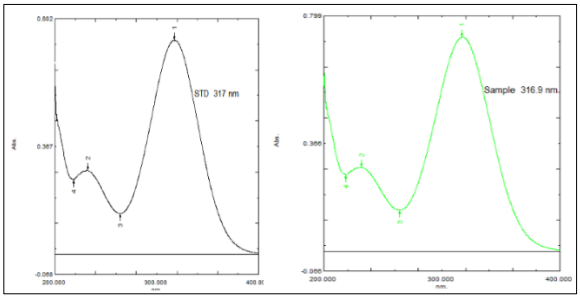

Fig 5: Tinidazole selectivity study

3.2.2 Linearity

A calibration curve was prepared by plotting the absorbance as a function of concentration of drug solution. The regression equation of the calibration curve was found to be y = 37.179*x* + 0.0176 The calibration curve is shown in (Fig 6) and represented in (Table 1). The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

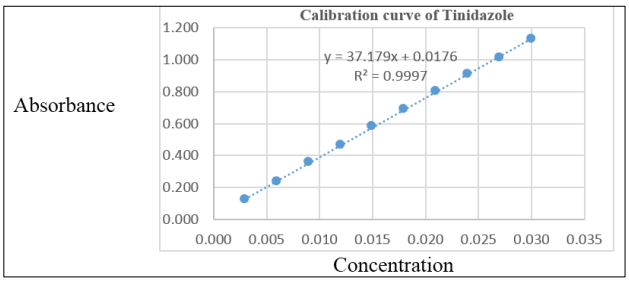

Fig 6: Concentration of Tinidazole in mg/mL

3.2.3 Lod and Loq

The limit of detection (LOD) was found to be 1.56 µg/mL and the limit of quantification (LOQ) was found to be 4.73 µg/mL, these parameters are summarized in table 1.

Table 1: Summary of quantitative parameters

Parameters	Results
Linear range(µg/mL)	3.0-30
Regression equation	y = 37.179x + 0.0176
Intercept	0.0176
Slope	37.179
Standard deviation	0.0176
Correlation coefficient (r)	0.9997
limit of detection, LOD (µg/mL)	1.56
Limit of quantitation, LOQ (µg/mL)	4.73

3.2.4 Accuracy and precision

The accuracy and precision of the method were similarly evaluated. The percentage relative error as accuracy was not exceed (1.224505%), intraday precision expressed in relative standard deviation did not exceed (0.711862%) and inter-day precision expressed in standard deviation which did not exceed 0.5, that indicating the high accuracy and precision of the method. The results of this study are compiled in (Table 2- 4) reflecting the usefulness of this method in routine analysis of the drug in quality control laboratories.

Table 2: Summary of quantitative parameters (accuracy)

Amount taken (%)	Amount Found (mg ml^{-1})	Recovery	Recovery error %
50	49.57491	99.14983	0.850173
50	49.38775	98.77549	1.224505
50	49.41416	98.82832	1.171676
100	100.7189	100.7189	-0.71885
100	99.31624	99.31624	0.683761
100	100.2355	100.2355	-0.23547
150	149.2783	99.51888	0.481123
150	150.8972	100.5981	-0.5981
150	149.8301	99.88672	0.113281

Table 3: Intraday precision results (Repeatability)

Amount taken (%)	Amount Found	Recovery	RSD %
50	49.46	98.91788	0.204816
100	100.09	100.0902	0.711862
150	150.00	100.0012	0.548643

Table 4: Inter-day precision results (Intermediate precision)

Tests	Analyst to Analyst		Day to Day	
	Analyst 1	Analyst 2	Day 1	Day 2
Test 1	100.14	99.59	100.7629	99.38913
Test 2	101.71	100.93	100.939	100.7941
Average	100.925	100.26	100.8509	100.0916
Average of all	100.6		100.5	
Relative standard deviation	0.5		0.5	

3.2.5 Robustness

The robustness of method was carried out; it was found that, small variation in the method variables (pH and λ_{max}) did not significantly affect the procedure; recovery values were shown in (Table 5).

Table 5: Summary of Robustness

Conditions	Optimum conditions	Interchanged Conditions	Recovery ± error
pH	6.8	6.7	98.68±1.32
		6.9	98.86±1.14
Max. abs.	317	315	98.63±1.37
		319	101.20±1.20

3.3 Application to dosage

Two dosage forms were considered to study the applicability of method on different dosage forms containing 500 mg tinidazole, the results shown in table 6.

Table 6: Determination of Tinidazole in tablets by the proposed method

Brand name and dosage form	Labeled claim (mg/tablet)	Amount found	Recovery (%±RSD)
Tinazol	500	504	100.8±0.801
Tinirem	500	501	100.2±0.210

Comparing this result with literature [5, 12, 13], this method was found to be simple, accurate, rapid and economical.

4. Conclusion

All the above factors lead to conclusion that, the proposed development spectrophotometric method for determination of Tinidazole is simple, accurate, precise, sensitive, robust and cost effective and can be applied as stability indicating method for estimation of tinidazole in quality control laboratories.

5. Acknowledgement

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