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Development and validation of stability indicating HPLC method for quantification of tinidazole

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RESEARCH ARTICLE

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ABSTRACT

A simple, precise, reproducible and economical HPLC method for estimation of tinidazole has been developed. The wavelength of 317 nm is selected as λ_{max} for tinidazole in phosphate buffer (pH = 6.8). Validation parameters were tested following International Conference on Harmonization (ICH) guideline. Tinidazole shows linearity at the selected wavelength and obeys Beer's law in the concentration range of 3.2-40.0 µg/mL with correlation coefficient of 0.9999. Recovery studies for tinidazole were performed and the percentage recovery was obtained in the range of 99.10-102.45% confirming the accuracy of the proposed method. The method showed good reproducibility and recovery with %RSD less than 2. Statistical validation of the data shows that the proposed method can be used as stability indicating method which successfully applied for the routine analysis of drug in commercial tablets.

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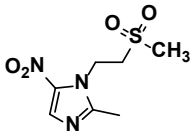
1. Introduction

Tinidazole (1-(2-ethyl sulfonylethyl)-2-methyl-5-nitroimidazole) is a nitroimidazole antiprotozoal agent effective against trichomonas vaginalis, entamoeba histolytica and giardia lambia infections. The nitro group of tinidazole is reduced by cell extracts of trichomonas. The free nitro radical generated as a result of this reduction is responsible for the antiprotozoal activity [1,2]. A literature survey revealed that only a few HPLC methods are available for the estimation of tinidazole [3-5].

Tinidazole is the subject of monograph in each of British Pharmacopeia (BP) and the United States Pharmacopeia (USP), either pharmacopeia recommends non-aqueous titration for determination of tinidazole [6]. Validation of analytical procedure as defined by International Conference Harmonization [7], is to demonstrate that it is suitable for its intended purpose. According to USP 2008 [8], 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. Tinidzole is degradated [9] as given in Scheme 2.



Scheme 1. Chemical structure of tinidazole.

A number of analytical methods for the quantitative determination of tinidazole in pharmaceutical preparations and biological fluids are known. Proposed methods mainly used are high performance liquid chromatography [10], high performance thin layer chromatography [11,12], gas liquid chromatography [13], packed column supercritical fluid chromatography [14], voltammetry [15,16], polarography [17], capillary electrophoresis [18], flow injection analysis [19], UV-spectrophotometry [20,21] and derivative UV-spectrophotometry [22].

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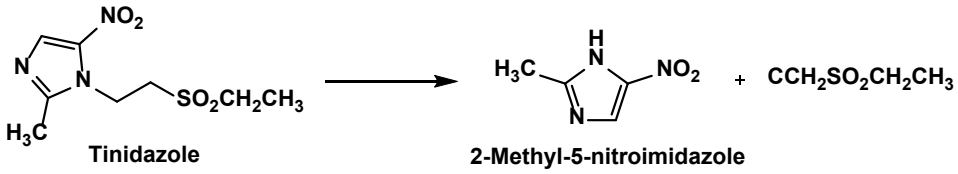

Scheme 2. Degradation of tinidzole.

As stated in BP pharmacopeia, the potentiometric titration is used for tinidazole analysis. The potentiometric titration process is time consuming and hazardous solvent are used, instead HPLC is simple and cost-effective method. The aim of this work is to develop and validate environment friendly a HPLC method for determination of tinidazole.

2. Experimental

2.1 Instruments and materials

The HPLC system consisted of a Shimadzu LC-20AB solvent delivery pump and a UV/VIS detector (Shimadzu Corporation, Tokyo, Japan). pH meter (Sartorius PP-20, USA) was used in pH and conductivity measurements. All chemicals were of analytical grade. Methanol is HPLC grade. Distilled water was used in all experiments. The standard of tinidazole (99.87%) was obtained from Azal Pharma Laboratories, Khartoum, Sudan. Tablet formulation Tinazol (General Medicines Company, Khartoum, Sudan), Protogen (Hayat Pharmaceutical Industries Co. PLC., Amman, Jordan) and Protozole tablets (Medical Union Pharmaceuticals, Abu-sultan, Ismailia, Egypt) were purchased from a local market with labeled amount 500 mg tinidazol.

2.2. Preparation of reagents and solutions

2.2.1. Standard stock solution of tinidazole

An accurately weighed amount 16.0 mg of tinidazole was dissolved in 50 mL of methanol to prepare a stock solution of concentration 0.32 mg/mL.

2.2.2. Working standard

Standard solution (1.0 mL) was transferred to 20 mL standard flask, completed to the mark with buffer solution which prepared as bellow, final concentrating obtained was 0.016 mg/mL.

2.2.3. 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 standard flask and completed to the mark with water, adjusted to pH = 6.8 by adding drops of 0.2 M NaOH which prepared by weighing 4.0 g, dissolved in water and completed to the mark (500 mL standard flask).

2.2.4. Preparation and selection of mobile phase

The preliminary isocratic studies on a reverse phase C18 column (Diameter: 4.6 mm, length: 250 mm, particle size: 5 µm) with different mobile phase combinations of 0.2 M phosphate buffer of pH = 6.8 and methanol were studied for simultaneous separation of the drug. The optimal composition of mobile phase determined to be a mixture of methanol: buffer (50:50, *v*:*v*) and filtered through 0.45 µm nylon filter.

2.2.5. Preparation of blank solution

A placebo (content of drug except tinidazole, 10.3 mg) which is equivalent to tinidazole standard weight was dissolved in 50 mL of methanol, 1.0 mL of this solution was transferred to 20 mL standard flask, completed to the mark with buffer.

2.3. Validation procedure

2.3.1. System suitability

System suitability study of the method was carried out by five replicate analysis of solution containing 100% target concentration of tinidazole. Various chromatographic parameters such as retention time, peak area, tailing factor and theoretical plate of the column were determined. The method was evaluated by analyzing these parameters.

2.3.2. Selectivity

The selectivity of the method was ascertained by injecting mobile phase, diluent, blank and standard drug to determine interference.

2.3.3. Linearity and range

To demonstrate the linearity for tinidazole, different aliquots, 0.2, 0.5, 1.0, 1.5, 2.0 and 2.5 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 0.0032 to 0.0400 mg/mL.

2.3.4. 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. Precision

For the precision study, 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 in the same day. Intermediate precision was assessed by comparing the assays on different days (2 days) and different analyst, relative standard deviation (RSD) calculated.

2.3.6. Robustness

The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions included ratio of mobile phase and maximum absorbance (±2 nm).

Table 1. System suitability testing results.

Parameters	Tinidazole
Retention time (min)	5.809
Peak area	1327274
Theoretical plate	7816.134
Tiling factor	1.43
Flow rate (mL/min)	0.50

Table 2. Selectivity study results.

Parameter	No. of theoretical plates	Tailing factor	Resolution	Response of main peak
Mobile Phase	(-) ve	(-) ve	(-) ve	(-) ve
Diluent	(-) ve	(-) ve	(-) ve	(-) ve
Blank	(-) ve	(-) ve	(-) ve	(-) ve
Standard solution	7816.134	1.434	0.000	1327274

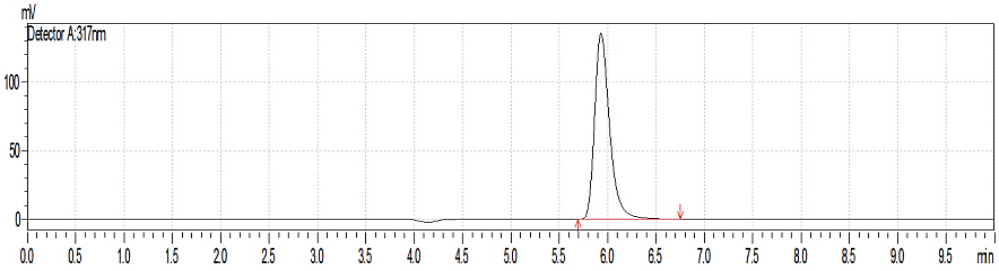

Figure 1. Chromatograms of tinidazole.

2.4. Stability study

2.4.1. Effect of some excipients on hydrolysis of tinidazole at 35, 45 and 55 °C

Four major expedients were considered for testing the interference with tinidazole; which are vivapore (micro crystalline cellulose), lactose monohydrate, sodium starch glycolate and povidone. Each excipient weight taken was 1% of tinidazole tablet weight at the different temperatures.

2.4.2. Stability to sun light

Standard solution was injected for six hours; the assay at each hour was calculated. Then, the degradation was evaluated for each 10 mins.

2.5. Applications of method to dosage form

The developed and validated HPLC method was applied for determination tinidazole dosage form. Tinazol tablet of 500 mg strength was evaluated. Ten tablets were powdered and powder equivalent to 500 mg of drug was weighed. The weighed sample was dissolved in methanol (50 mL), the suspension was sonicated for 10 min, filtered, and then 1.0 mL was taken in 20 mL volumetric flask to obtain 0.016 mg/mL final concentration (completed to the mark with buffer). Areas under the peak of the sample solution were recorded at determined λ_{max}, similarly, the recovery of Protogen and Protozole tablets containing 500 mg of tinidazole were evaluated.

3. Results and discussion

The objective of the present work was to develop a chromatographic method for determination of tinidazole and to validate the method by using various parameters.

3.1. Method optimization

Methanol was selected as a solvent. Many trials were carried out for the selection of a column and a mobile phase for the method development. After trials the column used in this method is C18 (250×4.6 mm, 5 µm) and the mobile phase is methanol: buffer (50:50, *v*:*v*). The wavelength was set at 317 nm as the drug showed good absorbance at this wavelength. The retention time of tinidazole was found to be 5.8 min. Run time was 10 minutes, flow rate 0.5 mL/min and injection volume was 20 µL. A typical chromatogram of the standard solution is shown in Figure 1 and the peak shape of drug was symmetrical. Table 1 is showing system suitability testing of tinidazole. The proposed method was validated as per international conference harmonization guideline.

3.2. Method validation

The proposed method was validated as per 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 that, the absence of interference, since there are no peaks appeared at the when blank, diluent and mobile phase were tested. Parameters shown in Table 2.

3.2.2. Linearity

A calibration curve was prepared by plotting peak area as a function of concentration of drug solution. The regression equation of the calibration curve was found to be $y = 67.557x + 29.45$. The calibration curve is shown in Figure 2 and represented in Table 3. 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.

Table 3. Summary of quantitative parameters.

Parameters	Value
Linear range (µg/mL)	3.2 - 40.0
Regression equation	$y = 67.557x + 29.45$
Standard deviation	29.45
Correlation coefficient (r)	0.9999
Limit of detection, LOD (µg/mL)	1.44
Limit of quantitation, LOQ (µg/mL)	4.36

Table 4. Summary of quantitative parameters (Accuracy).

Amount taken (%)	Amount Found (mg/mL)	Recovery	Recovery error %
50	50.05	100.10	-0.10
50	49.73	99.46	0.54
50	49.55	99.10	0.90
100	99.22	99.22	0.78
100	100.39	100.39	-0.39
100	102.45	102.45	-2.45
150	149.12	99.41	0.59
150	151.44	100.96	-0.96
150	150.85	100.57	-0.57

Table 5. Intraday precision results (Repeatability).

Amount taken (%)	Amount Found	Recovery	Relative standard deviation %
50	49.78	99.550	0.51
100	100.69	100.687	1.60
150	150.47	100.315	0.80

Table 6. Inter-day precision results (Intermediate precision).

Sample	Analyst to analyst		Day to day	
	Analyst 1	Analyst 2	Day 1	Day 2
Sample 1	101.42	99.05	100.82	99.32
Sample 2	100.75	99.26	101.4	100.79
Average	101.09	99.16	101.11	100.05
Average of all	100.1		100.5	
Relative standard deviation	1.4		0.7	

Table 7. Method robustness with various parameters.

Mobile phase (mL)			Max abs. (nm)		
Level	Used	Original	Level	Used	Original
+1	55:45	50:50	+1	319	317
0	50:50		0	317	
-1	45:55		-1	315	

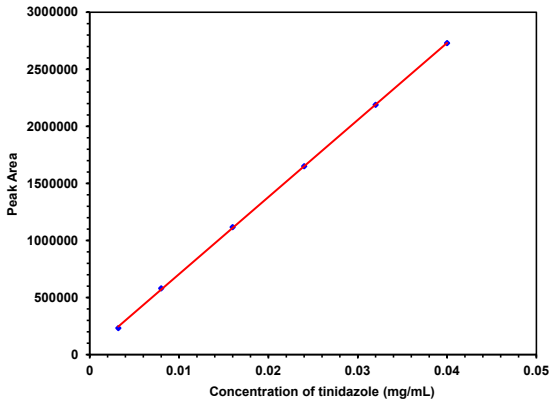

Figure 2. Calibration curve of tinidazole.

3.2.3. LOD and LOQ

The limit of detection (LOD) was found to be 1.44 µg/mL and the limit of quantification (LOQ) was found to be 4.36 µg/mL, these parameters are summarized in Table 3.

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 (1.29%), intraday precision expressed in relative standard deviation did not exceed (-0.31%) and inter-day precision expressed in standard deviation which did not exceed (1.40%), that indicates the high accuracy and precision of the method. The results of this study are compiled in Table 4-6 which reflecting the usefulness of this method in routine analysis of the drug in quality control laboratories.

3.2.5. Robustness

The robustness of method was carried out based on factorial design (Table 7); it was found that, small variation in

Table 8. Summary of robustness.

Conditions	Optimum conditions	Interchanged conditions	Recovery, %
Mobile phase ratio	50:50	45:55	98.620
(Buffer:Methanol)	50:50	55:45	99.214
Max absorbance	317	315	97.586
		319	101.720

Table 9. Effect of excipients on tinidazole %.

Temperature (°C)		35	45	55
Tinidazole %	Vivapore (Micro crystalline cellulose)	96.63	96.53	96.77
	Lactose monohydrate	97.20	95.50	95.37
	Sodium starch glycolate	94.29	94.60	93.93
	Povidone	101.77	98.32	94.54

Table 10. Degradation of tinidazole in sunlight.

Time (hour)	1	2	3	4	5	6
Tinidazole content (%)	16.24	9.27	0.70	0.12	0.09	0.08
Time (min)	10	20	30	40	50	60
Tinidazole content (%)	81.27	60.70	53.80	25.84	23.55	18.76

Table 11. Determination of tinidazole in tablets by the proposed method.

Brand name	Labeled claim (mg/tablet)	Amount found	Recovery% (±RSD)
Tinazol	500	495.08	99.02±0.98
Protogyn	500	496.79	99.36±0.64
Protozole	500	496.30	99.26±0.74

the method variables (mobile phase ration and λ_{max}) did not significantly affect the procedure; recovery values were shown in Table 8.

3.4. Stability study

3.4.1. Effect of excipients on tinidazole % at 35, 45 and 55 °C

According to Table 9, there was no drug degradation at different temperatures 35, 45 and 55 °C.

3.4.2. Stability in sun light

Tinidazole was obviously degraded in sunlight from the first hour, the assay is as follow, Then, the degradation was evaluated each ten minutes as shown in Table 10.

3.5. Application to dosage form

Three dosage forms were considered to study the applicability of method on different dosage forms containing 500 mg tinidazole, the results shown in Table 11. Comparing these results with literature [23,24], this method was found to be simple, accurate, economical and rapid.

4. Conclusion

The new HPLC method was developed and validated for simultaneous determination of tinidazole pharmaceutical dosage forms and assured the satisfactory precision and accuracy and also determining lower concentration of drug in its solid combined dosage form. The method was found to be simple, accurate, economical and rapid and they can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials and formulations. Compared to pharmacopeia method, it also proves to be environment friendly.

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Disclosure statement

Conflict of interests: The authors declare that they have no conflict of interest.
Author contributions: All authors contributed equally to this work.
Ethical approval: All ethical guidelines have been adhered.
Sample availability: Samples of the compounds are available from the author.

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