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Titrimetric and Spectrophotometric Determination of Tinidazole Tablets

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Abstract: A simple, sensitive, rapid, reproducible, economical and easily accessible method for the determination of tinidazole tablets is described. From the experiment carried out there is the need for recrystallisation of tinidazole tablet before it is analysized. Ultraviolet (UV) absorption analysis corresponds to the result obtained with non-aqueous titration. And the best solvent to be use for non-aqueous titration is acetic anhydride and crystal violet indicator can be used. The tablet must be extracted before analysis is carried out on it.

Key words: Tinidazole · spectrophotometry · nitroimidazole · antiprotozoal agents · trichomoniasis vaginalis

INTRODUCTION

The incidence of trichomonas infection among African women is on the increase [1, 2] and it is known that trichomoniasis facilitates the spread of HIV epidemic [3, 4]. One of the commonest drug used in the treatment of trichomoniasis is tinidazole [5, 6] the need to authenticate the quality of tinidazole using an affordable, rapid and reproducible method of analysis is desirable.

Various methods for the analysis of tinidazole have been reported among which are: Gas-liquid chromatography methods, spectrophotometric and absorptrometric assay [7-10]. Thin Layer Chromatography (TLC), Gas-liquid Chromatography (GLC) and high pressure liquid chromatography [11] and electrochemical method based on single-wall carbon nanotubes [12]. British Pharmacopoeia (BP) describes potentiometric and non-aqueous titration methods using perchloric acid as titrant [13]. United States Pharmacopoeia (USP) describes HPLC and non-aqueous titration methods for the assay of metronidazole only [14].

The aim of this research is to develop a simple, rapid, reproducible, economical and easily accessible method for the analysis of tinidazole tablets. The high incidence of faking and counterfeiting of drugs in our society and the enormous usefulness of tinidazole against *Trichomonas vaginalis* and *Helicobacter pyloris* warrant this study.

MATERIALS AND METHODS

Chemicals and reagents: Chloroform, methanol, acetone, distilled water, n-hexane, diethylamine, Formic acid, ethyl acetate Tinidazole tablets (Fasigyn® from Pfizer plc Nigeria). 0.1N perchloric acid, acetic acid, acetic anhydride, crystal violet, filter paper. All the chemicals and reagents were obtained from Sigma-Aldrich chemical.

Apparatus: Analytical thin Layer Chromatography (TLC) on silica gel 60 F_{254} plates from Merck (Darmstadt, Germany) was used. Thermometer, beaker test-tubes, sample tube, Volumetric flask, round-bottom flask, ultraviolet spectrophotometer, pipette, burette measuring cylinder, Electrothermal melting point apparatus, separating funnel, mortal and pestle were also used.

Extraction: The tablet was crushed into powder form and placed into a test tube. These were then extracted directly with 3x10 ml chloroform and the pooled chloroform extract was evaporated to dryness under reduced pressure. The crystals were then collected, dried and weighed.

Recrystallization: Recrystalization of the extract was done from hot methanol. The methanol was heated in a hot water bath, the crystals dissolved in the hot methanol; the solution was filtered using a glass funnel. The filtrate was thereafter cooled and the crystals were collected by filtration from the cold solvent. The crystals obtained where needle like and yellowish. The melting point of the extract was determined before and after the recrystallization.

Thin Layer Chromatography (TLC): The extract were dissolved in chloroform. Three solvent systems (as mobile phase) were used each time.

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System A-Chloroform: acetic acid (9.1)
B-Ethylacetate: methanol : Hexane (7:2:1)
C-Chloroform: diethylamine (9:1)

Ultra-violet spectrophometric (U.V) assay: 10 mg of the sample were weighed and dissolved in 10 ml of chloroform in a 10ml-volumetric flask to give a solution of 1mg/ml. A 1 in 10 dilution of this was made to give 100 ug/ml stock solution from which dilution of varying concentration of-0.05 ug/ml and 0.10 ug/ml, 0.15 ug/ml, 0.20 ug/ml, 0.25 ug/ml, 0.30 ug/ml and 0.40 ug/ml were prepared.

The corresponding absorbance was then measured from the UV spectrophotometer and graph of absorbance against concentration was then plotted as shown in Fig. 1. The dilution is shown in Table 1 and the absorbance data in Table 2.

Volumetric analysis

Standardization of 0.1N perchloric acid: About 0.2g of potassium hydrogen phthalate was accurately weighed, previously dried at 120° for 2 hours into a 250ml conical flask, 10ml of acetic anhydride was added and immediately a reflux condenser was attached. This was warmed until the salt dissolves, cooled and titrated with approximate 0.1N perchloric acid.

Non aqueous titration: Tinidazole (150 mg) was weighed and transferred to a clean dry conical flask and 50ml acetic anhydride was added. The content of the flask were

Table 1: Dilution table

Concentration at (ug ml^{-1})	Volume taken from stock solution (ml)	Volume taken from chloroform (ml)	Total volume
0.05	0.05	4.95	5 ml
0.10	0.10	4.90	5 ml
0.15	0.15	4.85	5 ml
0.20	0.20	4.80	5 ml
0.25	0.25	4.75	5 ml
0.30	0.30	0.70	5 ml
0.40	0.40	4.60	5 ml

Table 2: Absorbance data for tinidazole

Concentration (ug ml^{-1})	Absorbance at 314 um
Zero	0.042
0.05	0.258
0.10	0.416
0.15	0.548
0.20	0.895
0.25	1.076
0.30	1.095
0.40	1.797

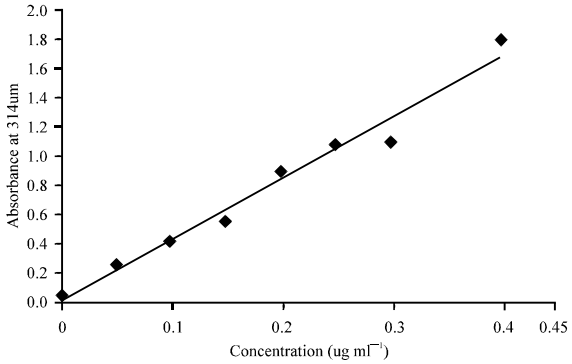

Fig. 1: A graph of absorbance against concentration

stirred to dissolve the drug. Then 2 drop of crystal violet indicator was added and the solution titrated with 0.1N perchloric acid, this titration was also performed using the powder (unextracted) tinidazole tablet. Each ml of 0.1N perchloric acid is equivalent to 24.72 mg of Tinidazole. See Table 3 for result.

Other solvent medium was also used for the tinidazole extract, which are acetone and acetic acid.

Note: The indicator was neutralized with 0.1N perchloric acid before the sample was added.

RESULTS

Introduction: The official recommendation for tablets weighing 250 mg or more is that the percentage deviation about the average weight should be±10% and that the number of tablet in the sample that may deviate by more than the above percentage i.e. for±5% should be one tablet and none for±10%. From the results of the tablet weighed the percentage deviation range is 0.1-0.8% which shows that the tablet weights are uniform and within acceptable level.

Total weight of powder tablet	= 2.80678 gm±0.0152
Total weight of coating materials of 5 tablet	= 0.11913 gm±0.0102
Weight of crystals formed from Extraction	= 1.4300 gm
Weight after recrystallisation	= 0.9615 gm
Melting point before recrystallisation	= 124-126°C
Melting point after recrystallisation	= 127-128°C

DISCUSSION

The melting point of crystal obtained before recrystallisation was 124-126°C which deviate appreciably from the literature value (127-128°C) but when it was recrystalised using methanol as solvent the melting point

Table 3: Results of the volumetric analysis

Solvent medium used	Volume of titrant used 0.1N perchloric acid (ml)	% purity w/w (SEM)
(a) For extract		
Acetone	8.70 ml (0.150 g)*	69.10%±2.01
Glacial acetic acid	9.40 ml (0.150 g)*	64.76%±1.05
Acetic anhydride	6.30 ml (0.130 g)*	6.10 ml (0.150 g)*
	8.00 ml (0.200 g)*	82.64%±0.96
	98.50%±1.18	100.12%±1.03
(b) For tablets (unextracted)		
Solvent medium used	Volume of titrant used 0.1N perchloric acid (ml)	% purity w/w (SEM)
Acetic anhydride	5.8 ml (0.180 g)*	7.5 ml (0.200 g)*
	7.7 ml (0.250 g)*	122.9%±2.18
	106.8%±1.95	130.0%±3.08
(c) Standardisation of 0.1N perchloric acid		
Solvent medium used	Volume of 0.1N perchloric acid used	
Acetic anhydride	9.90 (0.20 g)*	

The weight of the sample taken ( )

was 127-128°C which correspond to the literature value and was also sharp, so recrystallisation is necessary because it is purer.

The TLC analysis reveals the purity of the crystals because only one spot was seen both in daylight and UV lamp (254 nm) and the shape of the spot was oval for all the solvent system used.

The result obtained from the UV absorption analysis and the plot of absorbance versus concentration shows a linear relationship and the r-value obtained from the calculation was 0.5017, which also reveal that there is linear association between absorbance and concentration. The gradient obtained from the graph was 4.2; all this reveals the purity of tinidazole. This UV results were in good agreement with that of the non-aqueous method.

In the Non-aqueous titration method, the results obtained (i.e. % purity) from using different solvent i.e. acetone, glacial acetic and acetic anhydride were different and the best result was obtained with acetic anhydride and it also give a sharp colour change with crystal violet unlike the rest solvents. The percentage purity obtained with the extract using acetic anhydride were 98.5 and 100.12% w/w when 150 mg and 200 mg sample were used respectively and this can be said to be within official value but the percentage purity obtained for the tinidazole tablets (i.e. unextracted) were 106.8, 122.9, 130.0% w/w when 180, 200 and 250 mg sample were used. From this result it shows that the excipients have effect on the result obtained so the tablet cannot be analyse directly except it is recrystallized. With the result obtained from this work it shows that tinidazole tablets can be assayed using this simple, affordable, rapid and economical means of analysis described in this work whereby a more sophisticated methods e.g. HPLC is not available.

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