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DETECTION AND SPECTROPHOTOMETRIC DETERMINATION OF TINIDAZOLE

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ABSTRACT: A direct spectrophotometric method was developed by the authors for the quantitative determination of tinidazole in a pure form and also in other pharmaceutical formulations. The method was based on the diazotization reaction between nitro group of the drug sample, sulphanilamide and NEDA. In the present method, the reddish-purple colour dye formed, exhibited a maximum absorbance at 540nm. Beer's law was found to be obeyed in the range of 100-600µgmL^{-1}for tinidazole with detection limits of 0.04µgmL^{-1}. The present method was found to be precise, accurate for the qualitative and quantitative determinations.

Keywords : Spectrophotometry, tinidazole, 5-nitroimidazoles, quantitative determination

INTRODUCTION

5-Nitroimidazoles such as tinidazole and secinidazole are extensively used as anti amoebic, anti protozoal and anti bacterial drugs. The anti bacterial and anti trichomonal properties of the antibiotic azomycin led to the investigation of nitroimidazoles as anti parasitic agents. Although the amoebicidal properties of secinidazole were established, they were not clinically tested for quite some time.

Variation in the structure of metronidazole, principally to improve trichomonacidal activity and metabolic stability, led to the discovery of tinidazole. Tinidazole was found to be active against E. *histolytica* in vitro*;* cecalamoebiasis in rats, and hepatic amoebiasis in hamsters. Clinical tests have established tinidazole, in the treatment of intestinal and hepatic amoebiasis in humans.
Tinidazole was determined by titrimetry, potentiometry and HPLC methods. Indian Pharmacopoeia describes non-aqueous titration method, using perchloric acid as titrant and malachite green as indicator, for the assay of tinidazole. British Pharmacopoeia describes potentiometric and non-aqueous titration methods, using perchloric acid as a titrant.
In the literature, it was found that all the quantitative determinations were time consuming procedures, involving the reduction of nitro group followed by the addition of a chromogen. The Table 1. gives various reagents so far used, for the estimation of tinidazole and metronidazole with specific reagents, conditions and ranges of detection.
Most of the listed spectrophotometric methods in Table.1 , for the determination of tinidazole in the visible region, involve initial reduction, by treatment with Zn and HCl followed by the diazotization and coupling of the resulting amine. All these methods are less sensitive, involve tedious procedures, such as heating and extraction and involve utilization of costly reagents with an additional diazotization step. The present method is an attempt to overcome the above shortcomings of the existing procedures. The author's succeeded in developing a simple, rapid and accurate spectrophotometric procedure for the assay of tinidazole.

Table.1. Literature survey of the spectrophotometric determination of tinidazole and metronidazole.				
Reagents used	λmax in nm	Beer's law range in µg mL^{-1}	Critical experimental conditions involved	Reference
p-Dimethyl amino cinnam aldehyde	510	50 – 400 for MZ	Involves reduction with Zn-HCl and low sensitivity. Analysed only MZ.	3
4-Dimethyl amino benzaldehyde	550	10 – 100 for TZ	Involves reduction with Zn-HCl and low sensitivity. Analysed only TZ.	4
β-Naphthol	480	10 – 80 for MZ	Involves reduction with Zn-HCl and diazotisation and coupling with the cited reagent. Low sensitivity. Analysed only MZ.	5
Metol and $K_2Cr_2O_7$	720	2.4 – 24 for TZ	Involves reduction with Zn-HCl and the use	6
		1.6 – 16 for MZ	of buffer of pH 2.9 and colour formation, and its stability is pH dependent.	
NN-dimethyl-p-phenylenediamine and chloramine-T	540	4 – 36 for TZ 3 – 24 for MZ	Involves reduction with Zn-HCl and the use of buffer of pH 7 and colour formation and its stability is pH dependent.	7
Vanillin	412	10 – 50 for TZ	Involves reduction with Zn-HCl and heating for20 min with the reagent and cooling before absorbance measurement. Analysed only TZ.	8
Salicylaldehyde	380	20 – 70 for TZ	Involves reduction with Zn-HCl and low sensitivity. Analysed only TZ.	9
Bromocresol purple	618	2 – 24 for MZ	Involves extraction with $CHCl_3$ and use of buffer of pH 10.	10
Bromocresol green	654	2 – 22 for MZ	Involves extraction with $CHCl_3$ and use of buffer of pH 9.5.	-do- 11
NaOH and KCl	368	10 – 30 for TZ	Low sensitivity and involves heating at 100°C for 10 min.	12
Bromothymol blue	440	not given	Involves extraction with $CHCl_3$ and use of buffer of pH 4.4.	13
Methylbenzothiazolin-2-onehydrazine (MBTH)	500 and 490	1-32 for MZ 4-36 for TZ	Involves reduction with Zn-HCl and MBTH is a costly reagent.	14
N(1-naphthyl) ethylene diamine dihydrochloride (NEDA)	520 and 505	0.5 – 18 for MZ and TZ	Involves reduction with Zn-HCl and an additional step of diazotisation. Beer's law valid for low range of concentration.	15

METHODS AND MATERIALS

Reagents

Tinidazole tablets:

Ten tablets, tinidazole, of different pharmaceutical companies, were accurately weighed and ground to a fine powder. 500mg of such a sample was weighed and dissolved in 150ml of double distilled water. This mixture is heated to a temperature of 90° C for 90minutes. The cooled solution, after complete dissolution of the sample, was filtered through a Whatmann No 40 filter paper. The clear filtrate solution was made up to the mark in a 100ml volumetric flask and standardized [1,2].

0.5% sulphanilamide in 20 %(V/V) hydrochloric acid:

A stock solution of 0.5% sulphanilamide was prepared by dissolving an accurate amount of 0.5g of sulphanilamide in 20% hydrochloric acid, and the solution is made up to the mark using 20% hydrochloric acid in 100ml volumetric flask.

0.3% NEDA solution in 1 %(V/V) hydrochloric acid:

A stock solution of 0.3% NEDA was prepared by dissolving an accurate amount of 0.3g of NEDA in 1% hydrochloric acid. The solution was made up to the mark using 1% hydrochloric acid, in a 100ml volumetric flask.

All reagents used are of AnalaR quality.

Apparatus

An ELICO SL-177, Scanning Visible Spectrophotometer was used for all absorbance measurements, with a matched set of 1cm glass/ quartz cuvettes. Shimadzu-AUX 220- digital electronic balance was used for all weighing measurements. An ELICO LI-127- pH-meter was used for all pH measurements.

Recommended procedure for the determination of tinidazole and secinidazole:

An aliquot (2.0ml) volume of the drug sample of tinidazole was mixed with a 2ml of each 0.5% sulphanilamide and 0.3% NEDA reagents, to give an instantaneous, stable reddish- purple coloured product. The mixture was made up to 50ml, in a volumetric flask and the spectra of such a coloured product showed a λ_{max} at 540nm (Fig.1).

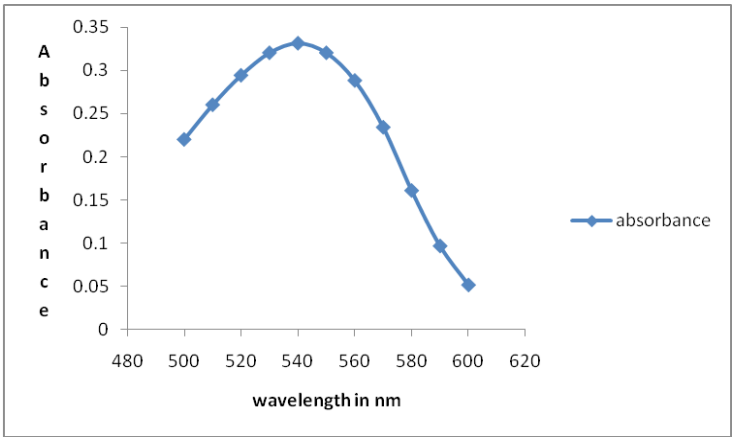

Fig.1 Absorption spectrum of the reddish-purple coloured product obtained by the reaction between tinidazole sulphanilamide and NEDA. The λ_{max} is 54o nm

For the determination of tinidazole, an aliquot volume of the sample solution was mixed with 2ml of each 0.5% sulphanilamide and 0.3% NEDA reagents, to give stable, instantaneous, reddish-purple coloured product. The mixture was made up to 50ml in a volumetric flask. The solution was taken in an optically matched cuvette of ELICO SL-177 spectrophotometer and the absorbances are measured at 540nm. The observed absorbance was compared with the standard curve (Fig.2). Beer's law was found to be valid over the range 100-600µgmL^{-1} for tinidazole (Fig.2).

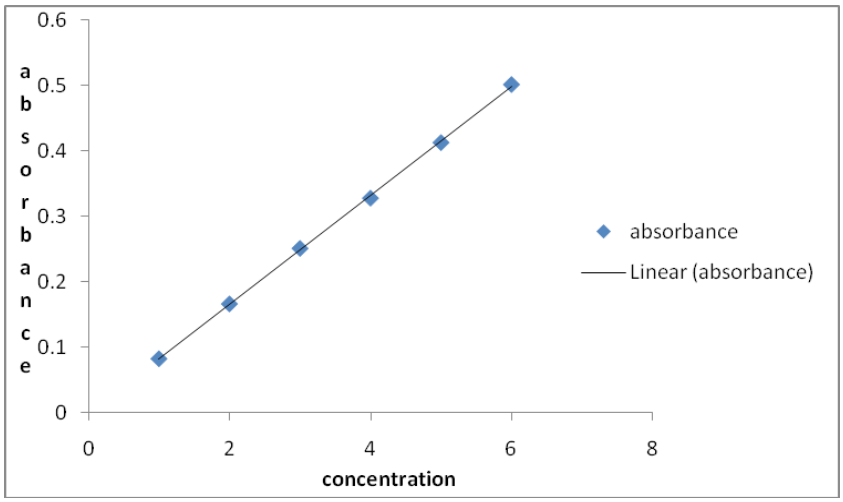

Fig.2 Calibration plot for the estimation of tinidazole with sulphanilamide and NEDA. Beer's law obedience is in the range of 100-600µgmL^{-1}

RESULTS AND DISCUSSION

The reddish purple colour obtained for tinidazole with sulphanilamide and NEDA was determined at a λ_{max} of 540nm. There is no overlap of spectra of other components used in the estimation. It was observed that the reaction was dependent on the pH as well as on the concentration of the reagents.The colour produced was found to be stable at a pH value of 3.5. The concentration of the reagents also has an appreciable effect on the colour produced. The reddish-purple colour of the product instantaneously was obtained and stable with 0.5%sulphanilamide and 0.3% NEDA solutions. This is found to be the optimum concentration. Hence the concentrations of the reagents were fixed as 0.5% sulphanilamide and 0.3% for NEDA. For each of the standard solution prepared, the absorbance measurements were recorded for every 30minutes and continued for 3 hours. The reaction product attained absorbance maximum within 30minutes and was found to be stable for more than 24 hours. Though the colour attained is instantaneous, it was found that the measurements taken earlier than 30minutes were inaccurate.

Beer's law was found to be valid over a range 100-600µgmL^{-1} for tinidazole. The molar absorptivity (**ε**) of tinidazole was found to be 3.386X10^2 cm^{-1} lit mole^{-1} . Detection limits (LOD) for tinidazole were found to be 0.04µgmL^{-1} . The limit of quantitation (LOQ) for tinidazole was 0.13µgmL^{-1}. The correlation factor for tinidazole was 0.9998. Relative standard deviation calculated for 10 measurements for each of the sample of drug was found to be well within standard limit prescribed, such as 1.51% for tinidazole. The calculated lower values of RSD indicate the good precision and reproducibility of the method. From the data, it was found that the LOQ values were 3.3 times greater than the LOD values. LOD is well below the lower limit of the Beer's law range. Commonly used excipients and other additives such as glucose, dextrose, lactose, starch, sodium alginate, talc, magnesium alginate, and magnesium stearate, and ascorbic acid were found to have no interference. The results were found to be accurate, precise and reproducible.

Table.2. Optical characteristics and validation data

Parameters	Tinidazole
λ_{max} (nm)	540
Beer's law limit (μgmL^{-1})	100-600
\Molar absorptivity (cm^{-1} lit $mole^{-1}$)	$3.386X10^2$
Stability (h)	> 24
Correlation coefficient, r	0.9998
t-test, p, CI (%)	0.0010, 4.57, 98
Relative standard deviation RSD*	1.51%
Limit of detection (μgmL^{-1})	0.04
Limit of quantification (μgmL^{-1})	0.13

* 10 replicate analysis of 200μgmL^{-1}

Table 3. Analysis for tinidazole and secinidazole formulations

Commercial formulations analyzed	PM#	SM@	RSD**
Timba 500mg (TZ)	100.2	100.0	1.5
Norfloxcin-Tinidazole-500mg	99.8	99.9	1.8
Tina (TZ)-300mg	98.7	99.8	1.7

Proposed method @Standard method ** Relative standard deviation

CONCLUSIONS

The solutions of tinidazole and secinidazole gave an instantaneous, stable reddish-purple coloured product with 0.5% sulphanilamide and 0.3% NEDA solutions. The λ_{max} for the reddish-purple coloured product was 540nm, with molar absorptivitites(**ε**) of $3.386X10^2$ cm^{-1} lit $mole^{-1}$ and $1.694X10^2$ cm^{-1} lit $mole^{-1}$ at 540nm. Beer's law was found to be valid over the range 100-600μgmL^{-1} for tinidazole. The determination of the drug samples was rapid, accurate and hence, recommended.

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