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Simple spectrophotometric determination of tinidazole in formulation and serum

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Abstract

A simple and rapid spectrophotometric method for the determination of tinidazole is presented. This method is based on the measurement of the absorbance of the signal at 368 nm yielded by bathochromic shift during alkaline hydrolysis of tinidazole in 0.1 N NaOH. The method is linear within the range of 1–30 μg ml^{-1}, and the detection and quantification limits are 0.07 and 0.25 μg ml^{-1}, respectively. The precision of the method, expressed as the relative standard deviation, is 0.19% for a tinidazole concentration of 15 μg ml^{-1}. The method was applied to the analysis of tinidazole in pharmaceutical formulations and serum.

Keywords: Tinidazole; Spectrophotometry; Serum; Formulations

1. Introduction

Tinidazole 1-[2-(ethylsulphonyl)ethyl]-2-methyl-5-nitroimidazole is an antimicrobial agent that like other 5-nitroimidazoles possesses selective activity against anaerobic or microaerophilic bacteria and protozoa [1]. It is used in the treatment of trichomoniasis in human beings.

For the measurement of nitroimidazole related compounds in human plasma, polarographic methods based on that described by Kane [2] have been used [3]. De Silva et. al. [4] described methods for the assay of *N*-substituted nitroimidazole in blood and urine which involved a preliminary separation of the unchanged drug by thin layer chromatography (TLC) before quantification by an absorptiometric, polarographic or gas chromatographic procedure. This TLC procedure was improved by Welling and Monro [5] where tinidazole was quantitated at the TLC stage by measuring the quenching of plate fluorescence. This method has been used for pharmacokinetic studies both in laboratory animals [6] and human beings [5,7]. More recently chromatographic methods were used for the assay of tinidazole in plasma and faeces using high performance liquid chromatography [8]. To our knowledge, at present, there is no officially accepted method for the determination of tinidazole.

In the spectrophotometric studies of pH variations in tinidazole solutions, a marked bathochromic shift from acidic to alkaline media was observed. This process of alkaline hydrolysis yields a new species with a maxima absorption at 368 nm of higher

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sensitivity and the disappearance of maximum absorption at 318 nm in acidic or neutral media. This reaction might be used for the determination of tinidazole in formulations and biological fluids by direct spectrophotometry. No former informations on this alkaline hydrolysis reaction was found.

In this work a new method for the determination of tinidazole based on its alkaline hydrolysis reaction is proposed. The method is applied to the determination of tinidazole in formulations and blood serum.

2. Experimental

2.1. Reagents

Tinidazole obtained from Sigma (St. Louis, MO) was used. Standard solutions of tinidazole were prepared by dissolving the appropriate amount in deionized water. Sodium hydroxide solutions of different concentrations were prepared from NaOH, Merck (Darmstadt), and standardized against potassium phthalate acid. All other chemicals were of analytical reagent grade.

2.2. Apparatus

A Spectronic 3000 Diode Array Milton Roy spectrophotometer with 0.35 nm resolution coupled to a 486 PC was used. A User Data version 2.01 Milton Roy software for spectral data acquisition, storage and manipulation of spectrophotometric data was used.

2.3. Procedure

2.3.1. Determination of tinidazole

Samples were prepared in 25 ml calibrated flasks containing suitable aliquots of tinidazole, 5 ml of 0.5 N NaOH solution, 5 ml of 0.5 N KCl solution and diluted with deionized water to the mark. Then, the flasks were immersed in a boiling water bath for 10 min. The flasks were cooled and the absorption spectra recorded against a reagent blank. The absorbance of the samples were measured at a wavelength of 368 nm. The content of tinidazole was determined by using the appropriate calibration graph.

2.3.2. Determination in formulations

2.3.2.1. Tablets. Ten tablets were carefully pulverized and a representative amount was accurately weighed, then carefully stirred in deionized water for 30 min, filtered, washed and diluted to a known volume with deionized water. Suitable aliquots of the solution were used to carry out the analysis according to the above described procedure.

2.3.2.2. Suspension. Suitable aliquots were taken, diluted with deionized water and filtered. The clear solution obtained was diluted to a known volume with water. For the assay, appropriate amounts of this solution were taken and treated according to the described procedure.

2.3.2.3. Determination in serum. Aliquots of 1.0 ml of serum were deproteinized by the addition of 2.5 M $HClO_4$ solution. The serum, protein free, was separated by centrifugation, its pH was adjusted to 6.0, and the above mentioned procedure for determination of tinidazole was followed.

3. Results and discussion

As mentioned in Section 1, tinidazole solutions are sensible to pH variations from acidic to alkaline media (Fig. 1). In alkaline media, tinidazole under-

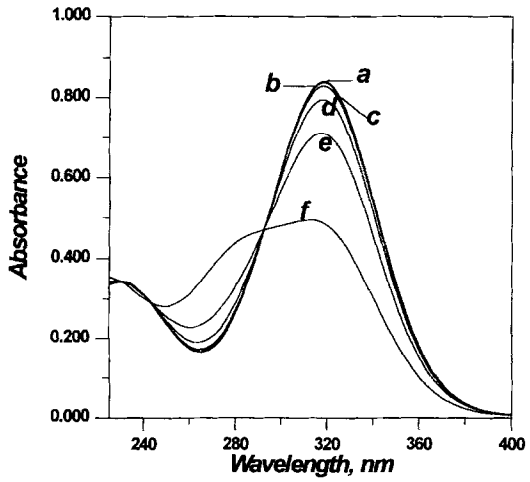

Fig. 1. Influence of pH on the absorption spectra of tinidazole. pH value, (a) 7.36; (b) 5.35; (c) 3.34; (d) 2.75; (e) 2.25 and (f) 1.75.

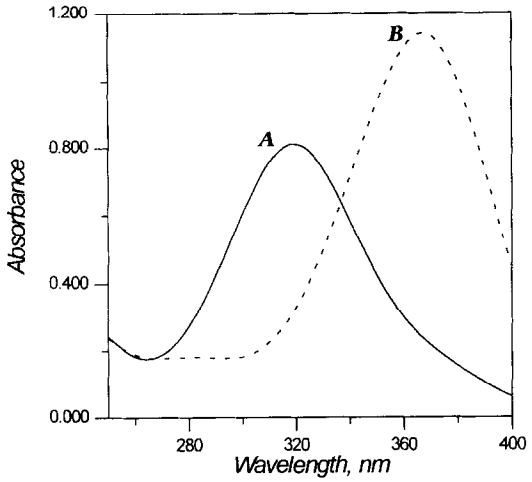

Fig. 2. Absorption spectra of tinidazole. (A) before and (B) after hydrolysis. Without hydrolysis and hydrolyzed with 0.1 N NaOH solution; heating time, 10 min in a boiling water bath.

goes a hydrolysis reaction which produces a new species with maximum absorption wavelength at 368 nm, of higher sensitivity and very stable. The absorption spectra of tinidazole before and after the hydrolysis are shown in Fig. 2. The signal at 318 nm disappears gradually with the time and the new signal appears as the reaction proceeds. This hydrolysis reaction is accelerated by temperature.

The influence of several parameters on the alkaline hydrolysis reaction of tinidazole was studied.

Temperature and time of heating were first considered. This study was done by fixing 0.5 N NaOH concentration. Samples were prepared containing 20 μg ml^{-1} of tinidazole, 0.5 N NaOH, 0.1 N KCl and deionized water to complete the volume. Separate samples were immersed in a boiling water bath for 5, 10 and 15 min respectively, then cooled and the absorbance measurements at 368 nm were taken at time intervals of 5 min for 45 min. In all cases we obtained a maximum absorbance at time zero, which remained constant throughout the 45 min period. These results show that the reaction proceeds completely with a heating time of 5 min and a 0.5 N NaOH concentration.

The effect of the sodium hydroxide concentration on the reaction was approached by preparing samples in 25 ml calibrated flasks with 20 μg ml^{-1} of tinidazole, 2.5 ml of 1 M KCl solution, various amounts of 1.0 N NaOH solution and deionized water for dilution to the mark. The samples were immersed in a boiling water bath for 5 and 10 min respectively, cooled and the absorbance measured after each 5 min during 20 min. Results obtained show that hydrolysis finished within 5 min of heating when the NaOH concentration was 0.4 N, while for lower concentration values, absorbance continues increasing with time. Nevertheless, heating for 10 min, the hydrolysis reaction gets to completion when the NaOH concentration was 0.1 N. This was observed by the complete disappearance of the first peak at 318 nm and the appearance of the new peak at 368 nm that remains unchanged thereafter. A 0.1 N NaOH concentration was chosen for subsequent experiments. The influence of the alkali type on the reaction was studied by using potassium hydroxide solutions as the same concentrations as sodium hydroxide. A better response was observed with sodium hydroxide.

The influence of the ionic strength upon the reaction was studied by preparing samples in a similar way as the preceding study, with variable amounts of 2 M KCl solution. No significative variations were observed in the variations of ionic strength in the 0.1–0.5 M KCl range.

3.1. Tinidazole calibration graph

Under the optimum conditions established, a calibration graph for tinidazole was produced. Good linearity was obtained within a concentration interval of 1.0 to 30.0 μg ml^{-1}. The linear regression equation was $A=(0.0479\pm0.00037)\ C_{tin}+0.00097\pm0.00011$ ($r=0.9999$), the relative standard deviation (rsd, %) value obtained from the analysis of 11 samples with 15 μg ml^{-1} of tinidazole was 0.19, with a relative error of medium value (%E) 0.43, detection limit (LD) 0.07 ppm and determination limit (LQ) [9] 0.25 ppm.

3.2. Applications

The proposed method for tinidazole analysis has been applied to the determination of this compound in pharmaceutical formulations and serum. The results obtained are summarized in Tables 1 and 2.

Table 1
Determination of tinidazole in pharmaceutical formulations

Pharmaceutical formulation	Tinidazole claimed (mg)	Tinidazole found [a] (mg)	Tinidazole found [b] (mg)
Estovyn-t (tab)	500	496.16±0.75	497.92±1.62
Estovyn-t (susp)	67	66.33±0.49	66.72±0.54
Fasigyn (tab)	500	495.26±0.84	496.84±1.89

[a] Each value is mean of three determinations.
[b] Standard addition method.

Table 2
Determination of tinidazole in blood serum

Tinidazole added in serum (μg ml^{-1})	Tinidazole found [a] (μg ml^{-1})	Recovery (%)
5.0	4.76	95.20±1.89
8.0	7.69	96.13±1.46
10.0	9.79	97.90±0.95

[a] Each value is mean of five determinations.

In order to investigate the interference of excipients present in formulations, the standard addition method was applied. The results show a satisfactory recovery of tinidazole and agree well with the amounts stated by the manufacturer.

In the analysis of serum samples spiked with different amounts of tinidazole, the recovery was over 93%. It is worth to note the fact that the method can be applied directly without previous separation of tinidazole.

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