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ESTIMATION OF TINIDAZOLE IN TABLETS BY RP–HPLC METHOD

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ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the estimation of tinidazole in its pure from as well as in tablet dosage forms. Chromatography was carried out on an ODS column using a mixture of acetonitrile and potassium dihydrogen phosphate (35 : 65 v/v) as the mobile phase at a flow rate of 1.0 mL/min. The detection of the drug was monitored at 302 nm using omeprazole as an internal standard. The retention time of the drug was found to be 2.92 min. The method produced linear responses in the concentration range of 2 to 12 µg/mL of tinidazole. The method was found to be reproducible for analysis of the drug in tablets.

Key words: Tinidazole, Estimation, Tablets, HPLC.

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]. The authors now propose a new validated, sensitive and reproducible HPLC method for the determination of tinidazole. The applicability of this method in determining the drug in commercial dosage forms was also studied.

EXPERIMENTAL

Chromatographic conditions

A Shimadzu LC-2010 CHT high-performance liquid chromatographic instrument

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provided with a Shimadzu LC 2010 C series HPLC pump and a SIL LC 2010 C series auto sampler equipped with a 20 µL sample loop was employed in the study. A Kromasil ODS reverse phase column (250 mm x 4.6 mm; 5 µ) was used for the separation. Detection was done using an SPD LC 2010 C dual absorbance detector and the output signal was monitored and integrated using Shimadzu CLASS-VP Version 6.12 SPI software.

HPLC grade acetonitrile (Qualigens) and potassium dihydrogen phosphate (AR grade, Qualigens) were used for preparing the mobile phase. A freshly prepared 35 : 65 v/v mixture of acetonitrile and potassium dihydrogen phosphate was used as the mobile phase. The solvents were filtered through a 0.45 µ membrane filter and sonicated before use. Enertech ultrasonicator was used for this purpose. The flow rate of the mobile phase was maintained at 1 mL/min. The column temperature was maintained at 25^0C. The detection of the drug and internal standard was carried out at 302 nm.

Drug and internal standard solutions

A pure sample of tinidazole (M/s East India Pharmaceutical Works Ltd, Kolkata) was used as the reference standard in this study. About 100 mg of tinidazole was weighed accurately and transferred into a 100 mL volumetric flask and dissolved in 50 mL of the mobile phase. The solution was sonicated for 20 min and then the volume made up with a further quantity of the mobile phase to get a 1 mg/mL solution. Subsequent dilutions of this solution ranging from 2-12 µg/mL were made in 10 mL volumetric flasks after addition of 1.0 mL of omeprazole solution (200 µg/mL) as an internal standard to each dilution. Each dilution was injected five times into the column (20 µL) and the corresponding chromatograms were obtained. From these chromatograms, the ratio of the area under the peak of the drug to that of the internal standard for each dilution was calculated. The regression of the drug concentrations over the ratios was computed. This regression equation obtained was used to estimate the amount of tinidazole in pharmaceutical dosage forms.

Solutions containing 5 to 15 µg/mL of tinidazole were subjected to the proposed HPLC analysis to check the inter-day and intra-day variation of the method by adding known amounts of tinidazole to the pre-analyzed samples and then analyzing them by the proposed method.

Estimation of tinidazole in tablets

Two commercial samples of the tablets containing the drug (Tini of Kopran and Fasigyn of Pfizer) were chosen for testing the suitability of the proposed method to estimate tinidazole in tablets. For this, twenty tablets were taken and made into a fine powder. An

accurately weighed portion of this powder equivalent to 100 mg of tinidazole was transferred into a 100 mL volumetric flask and mixed with 50 mL of the mobile phase. The contents of the flask were allowed to stand for 6 hrs with intermittent sonication to ensure complete solubility of the drug and then filtered through a 0.45 µ membrane filter. From the filtrate, different aliquots were taken in separate 10 mL volumetric flasks. These solutions were spiked with a suitable volume of the internal standard solution, such that the concentration of the internal standard in each solution was 200 µg/mL. The contents of the flasks were made up to the volume with the mobile phase and mixed well. Twenty micro liters of each of these solutions was then injected five times into the column. The mean peak area ratios of the drug to the internal standard of five such determinations were calculated and the drug content in the tablets was quantified using the regression equation obtained for the pure sample.

RESULTS AND DISCUSSION

The aim of this study was to develop a simple, rapid, accurate and precise HPLC method for the analysis of tinidazole in bulk and tablet dosage forms. A mixture of acetonitrile and potassium dihydrogen phosphate in 35 : 65 v/v proportion was proved to be the most suitable of all combinations since the chromatographic peaks were better defined and resolved and almost free from tailing. Omeprazole was chosen as the internal standard because it showed better peak shape and peak location as compared to other internal standards under the above mentioned chromatographic conditions. The retention times obtained for tinidazole and the internal standard were 2.92 and 4.70 min, respectively. The chromatogram is shown in Fig. 1.

Each of the samples was injected five times and the same retention times were observed in all the cases. The ratios of the peak areas of tinidazole to those of the internal standard for different concentrations taken up were calculated and the average value for five such determinations are shown in Table 1. The peak areas of both; the drug and the internal standard, were reproducible as indicated by low coefficient of variation. A good linear relationship ($r = 0.998$) was observed between the concentration of tinidazole and the respective ratios of peak areas in the concentration range of 2 to 12 µg/mL of the drug. The linearity curve was constructed and its regression coefficient is $Y = 0.073 x + 0.013$ (where Y is the ratios of peak areas of the drug to that of internal standard and x is the concentration of tinidazole). When tinidazole solutions containing 5 10 and 15 µg/mL were analyzed by the proposed method for finding out the intra- and inter-day variations in the recoveries, a low coefficient of variation in the results was observed as shown in Table 2. This shows that the present HPLC method is highly precise. The amounts of tinidazole obtained from the

pre-analyzed samples containing known amounts of added drug are shown in Table 3. About 99.89% of tinidazole could be recovered from the pre-analyzed samples indicating high accuracy of the proposed method.

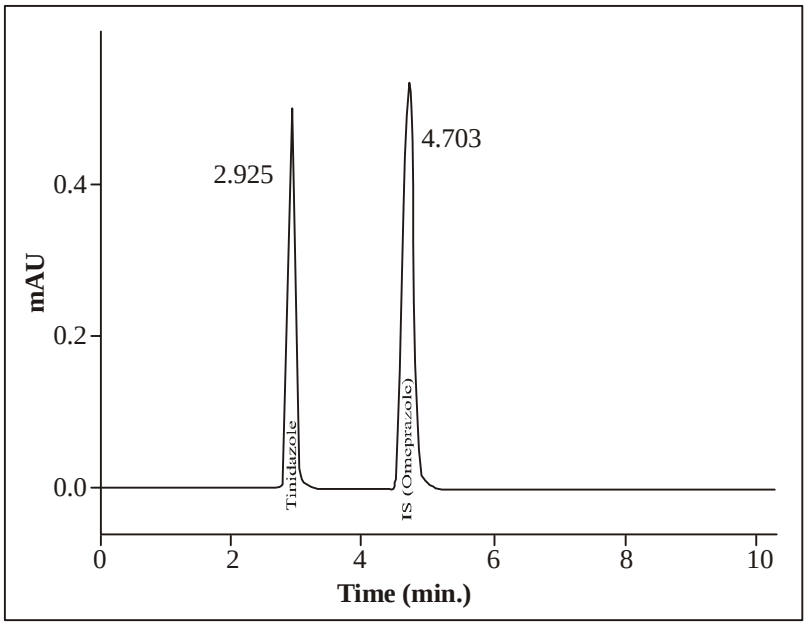

Fig. 1: A typical chromatogram showing the separation of tinidazole

Table 1: Calibration of the proposed method

Concentration (µg/mL)	Mean peak area ratio (n = 5)	Coefficient of variance (%)
2	0.153	0.002
4	0.304	0.002
6	0.456	0.006
8	0.610	0.012
10	0.761	0.015
12	0.875	0.011
Regression equation from 2-12 (µg/mL) : Y = 0.073x + 0.013 (r = 0.998)		

Table 2: Precision of proposed method

Amount of tinidazole taken (µg/mL)	Amount of tinidazole (µg/mL) obtained			
	Intra-day		Inter-day	
	Mean (n = 5)	RSD %	Mean (n = 5)	RSD %
5	4.974 ± 0.049	0.985	4.968 ± 0.089	1.791
10	9.942 ± 0.080	0.802	9.920 ± 0.120	1.210
15	14.926 ± 0.063	0.422	14.898 ± 0.121	0.812

Table 3: Recovery data of tinidazole

Amount of drug (µg) added to pure drug/ formulation	Recovery from drug solution		Recovery from tablets	
	Mean (± SD) amount (µg) found (n = 5)	Mean (± SD)% recovery (n = 5)	Mean (± SD) amount (µg) found (n = 5)	Mean ± (SD)% recovery (n = 5)
300	299.920 ± 0.130	99.970 ± 0.030	299.916 ± 0.109	99.972 ± 0.034
500	499.940 ± 0.110	99.990 ± 0.020	499.940 ± 0.112	99.988 ± 0.022

The drug content in the tablet was quantified by using the proposed analytical method. The tablets were found to contain an average of 99.98% of the labeled amount of the drug. The low coefficient of variation indicates the reproducibility of the assay of tinidazole in dosage forms. It can be concluded that the proposed HPLC method is sufficiently sensitive and reproducible for the analysis of tinidazole in pharmaceutical dosage forms within a short analysis time. The method was duly validated by evaluation of the required parameters.

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