Estimation of Tinidazole in Human Plasma by Reverse Phase HPLC Method

Y.S.R. KRISHNAIAH*, Y. INDIRA MUZIB, P. BHASKAR and V. SATYANARAYANA
Department of Pharmaceutical Sciences
Andhra University, Visakhapatnam-530 003, India

A rapid and sensitive high-performance liquid chromatographic method was developed for the estimation of tinidazole in human plasma. Varying amounts of tinidazole (10 to 800 ng), fixed quantity (2 μg) of mebendazole (internal standard) and 0.2 mL of 20% perchloric acid were added to blank human plasma (0.5 mL). The mixture was vortexed for 5 min and centrifuged at 3,000 rpm for 10 min and the supernatant liquid was separated and filtered. Twenty microliters of the resultant filtrate was injected into a reverse phase C-18 column using a mobile phase consisting of acetonitrile and water (consisting of 0.4% triethylamine and pH 3.6 adjusted with 5%orthophosphoric acid) in the ratio of 44:56 v/v at a flow rate 0.8 mL/min. The eluents were monitored at 254 nm. The calibration curve was linear in the range of 10–800 ng/0.5 mL of plasma. Due to its simplicity, sensitivity, high precision and accuracy, the proposed HPLC method may be used for biopharmaceutical and pharmacokinetic evaluation of tinidazole formulations.

Keywords: Tinidazole, Estimation, Plasma, HPLC.

INTRODUCTION

Tinidazole is a 5-nitro imidazoline derivative active against a wide variety of anaerobic protozoal parasites and bacteria[1]. It is highly active against *Entamoeba histolytica*, *Trichomonas vaginalis* and *Giardia lamblia*. Tinidazole is converted to the active metabolite by reduction at the 5-nitro position. The active metabolite causes DNA damage in pathogens[1]. Several analytical methods have been reported for the estimation of tinidazole in plasma including HPLC and gas chromatography[2–4]. The gas chromatographic method may require derivatisation and the process is considered tedious[2]. But some of the reported HPLC methods utilized special columns for the estimation of tinidazole in biological samples and the run time is more[3,4]. The HPLC methods are simple, sensitive, precise and highly accurate, and require small quantity of sample. However, the HPLC methods using the most commonly available columns and consuming less quantity of solvents are preferred. The present study describes a rapid method for the determination of tinidazole in human plasma by using RP C-18 column.

EXPERIMENTAL

Tinidazole and mebendazole were gift samples from M/s. East India Pharmaceuticals Limited, Calcutta, India and M/s. CIPLA Ltd, Bangalore, India. Acetonitrile and water used were of HPLC grade.Triethylamine,orthophosphoric acid and perchloric acid used in the study were of AR quality (Qualigens).

Instrumentation A gradient HPLC (Shimadzu HPLC Class VP series) with two LC-10AT VP pumps, variable wavelength programmable UV/Vis detector SPD-10A VP, CTO-10AS VP column oven (Shimadzu), SCL-10A VP system controller (Shimadzu), a disposable guard column LC-18 (Pelliguard™, LC-18, 2 cm, Supelco, Inc., Bellefonte, PA.) and RP C-18 column (150 mm × 4.6 mm I.D., particle size 5 μm, Flexit Inc., Pune, India) was used. The HPLC system was equipped with the software "Class-VP series version 5.03 (Shimadzu)".

HPLC conditions: The mobile phase components, acetonitrile and water (pH adjusted to 3.56 with 0.4% triethylamine and 5% orthophosphoric acid) were filtered before use through 0.2 μm membrane filter and pumped from the solvent reservoir in the ratio of 44 : 56 v/v to the column at a flow rate of 0.8 mL/min which yielded a column back-pressure of 120–137 kg/cm^2. The run time was set at 10 min, the column temperature was maintained at 40°C and volume of each injection was 20 μL. Prior to injecting solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the systems. The detector sensitivity was set at 0.0001 a.u.f.s and eluents were monitored at 254 nm.

Method: Six sets of plasma samples with varying drug concentrations were prepared by spiking drug-free plasma with an appropriate volume (100 μL) of tinidazole solution so as to obtain a concentration range of 10 to 800 ng/0.5 mL of plasma along with 100 μL of 2g/0.5 mL of mebendazole (internal standard) solution.

An aliquot of plasma (0.5 mL) was accurately measured into a 10 mL glass tube with a teflon-lined cap, followed by the addition of 100 μL of 2 g/0.5 mL of mebendazole (internal standard) solution and 0.2 mL of 20% perchloric acid. The mixture was vortexed for 5 min to ensure complete mixing of the contents and then centrifuged for 10 min at 3,000 rpm. The clear supernatant liquid was filtered through 0.2 μm membrane filter and twenty microlitres of filtrate was injected into reverse phase C-18 column and the eluents were detected at 254 nm. The peak areas of tinidazole and internal standard were recorded. The ratio of peak area of tinidazole to that of internal standard (mebendazole) was calculated, and the regression of the peak area ratio over the plasma concentration of tinidazole was calculated using the least squares method of analysis.

Precision: Aliquots of blank plasma (500 μL) were spiked with 100 μL of 2g/mL of internal standard and of tinidazole solutions (100 μL) so as to yield concentrations of 50, 100, 200 and 800 ng. Each plasma sample was extracted, as described above, and injected into the HPLC column (n = 5). Each sample was prepared in triplicate on three consecutive days, and were injected in to the HPLC column (n = 5) to observe the precision of the method.

Accuracy: The preanalysed plasma samples containing 100 ng/ 0.5 mL were added with known quantity of tinidazole (50 or 100 ng/0.5 mL) and subjected to

the proposed HPLC method, in triplicate. The difference in the measured concentration and that of the added quantity (50 or 100 ng/0.5 mL) was expressed in terms of per cent recovery.

RESULTS AND DISCUSSION

The run time of the method was set at 10 min. Tinidazole and mebendazole appeared on the chromatogram at 4.71 min and 6.62 min respectively (Fig. **1**). When the same drug solution was injected 6 times, the retention time of the drug and internal standard were same. This indicates that the proposed HPLC method is rapid, which in turn shows that the method consumes fewer amounts of expensive HPLC solvents. Table-1 shows the mean peak area ratios of tinidazole solutions for 6 such determinations. When the concentration of tinidazole and its respective peak area ratios were subjected to regression analysis by least squares method, a high correlation coefficient was observed ($r = 0.9998 \pm 0.059$) in the range of 10 to 800 ng/mL only. The regression of tinidazole concentration over its peak area ratio was found to be $Y = 0.0109 + 0.00076X$ where 'Y' is the peak area ratio and 'X' is the concentration of tinidazole. This regression equation was used to estimate the amount of tinidazole in plasma or in validation study (precision and accuracy).

TABLE- 1
CALIBRATION AND PRECISION OF THE HPLC ASSAY

Amount of tinidazole added to 0.5 mL human plasma (ng)	Peak-area ratio*	C.V. (%)
0	0	0
10	0.0232	1.21
20	0.0256	0.73
40	0.0438	0.98
100	0.0909	0.28
200	0.1630	0.31
400	0.3152	0.79
600	0.4660	1.86
800	0.6182	0.26

*Mean of six determinations
Regression equation: $Y= 0.01093 + 0.00076X$ ($r = 0.9998$)

The present HPLC method was also validated for intra- and inter-day variation. When a known amount of drug solution (100 ng) was added to preanalysed plasma samples (50 and 100 ng/0.5 mL), there was a high recovery (98.98%) of tinidazole indicating that the proposed method is highly accurate. To assess the precision,

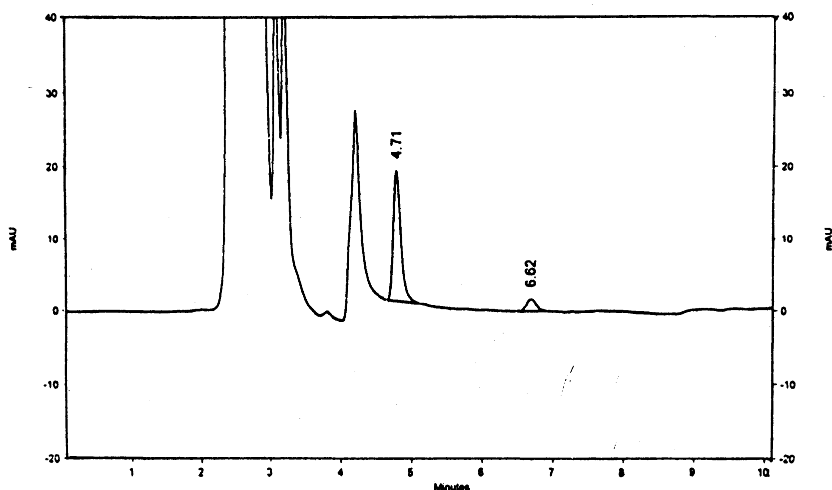

Fig. 1. A typical chromatogram of tinidazole in human plasma

the tinidazole solution (50, 100, 200 and 800 ng/mL) was added to the drug-free plasma along with internal standard solution. The plasma was treated as per the procedure described above, and the resultant filtrate was repeatedly injected on the same day and on three different days. The coefficient of variation (CV) in the peak area ratio for five replicate injections was found to be less than 2%. Also, the inter-day variation (3 days and five injections) was found to be less than 1.8%. Thus, the results show that this HPLC method is highly reproducible, simple, accurate and less time consuming than the reported methods[2-4].

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