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Reverse-Phase HPLC Method for the Estimation of Ornidazole in Human Plasma

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A high-performance liquid chromatographic method was developed for the estimation of ornidazole in human plasma. Varying amounts of ornidazole drug solution (10 to 800 ng) and 5 mL of ethyl acetate were added to blank human plasma (0.5 mL). The mixture was vortexed for five minutes and centrifuged at 3,000 rpm for 10 min and organic layer was separated and dried under vacuum evaporation .The residue is reconstituted with 0.3 mL of methanol. Twenty microliters of this solution was injected into a reverse phase C-18 column using a mobile phase consisting of methanol and triply distilled water (consisting of 0.4% triethylamine and pH adjusted to 3.6 with 5% orthophosphoric acid) in the ratio of 50 : 50 v/v and the eluents were monitored at 254 nm. The method was statistically validated for its linearity, precision and accuracy. The intra- and inter-day variation was found to be less than 2% indicating that the method is highly precise. Due to its simplicity, sensitivity, high precision and accuracy, the proposed HPLC method may be used for estimation of ornidazole in plasma.

Key Words: Ornidazole, Estimation, Plasma, RP-HPLC.

INTRODUCTION

Ornidazole is active against a wide variety of anaerobic protozoal parasites and bacteria[1]. It is highly active against *Entamoeba histolytica*, *Trichomonas vaginalis* and *Giardiasis*. Ornidazole is converted to the active metabolite by reduction at the 5-nitro position and the active metabolite causes DNA damage in pathogens[1]. Several analytical methods have been reported for the estimation of ornidazole in plasma including HPLC and gas chromatography[2-5]. The gas chromatographic method may require derivatisation and the process is considered tedious[2]. Some of the reported HPLC methods require ECD detection which requires passivation and so these are also consideed tedious[2]. 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 are preferred. In the present study a sensitive, accurate and precise HPLC method has been developed for estimation of ornidazole in human plasma by using RPC-18 column and simple UV detection.

EXPERIMENTAL

Ornidazole was a gift sample from M/s Aristo Pharmaceuticals Limited, Daman, India. Methanol and water used were of HPLC grade (Qualigens).

Triethylamine and orthophosphoric acid used in the study were of AR grade and supplied by M/s S.D. Fine-Chem Limited, Mumbai, India.

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: Both methanol and water (containing 0.4% triethylamine and pH adjusted to 3.6 with 5% orthophosphoric acid) were filtered through 0.2 μm membrane filter, and pumped from the solvent reservoir in the ratio of 50 : 50 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 column temperature was maintained at 40°C. The volume of each injection loop was 20 μL. The detector sensitivity was set at 0.0001 a.u.f.s and eluents were monitored at 254 nm

Procedure: Five sets of plasma samples with varying drug concentrations were prepared by spiking drug-free plasma with an appropriate volume (100 μL) of a known amount of ornidazole so as to obtain concentration range of 10 to 800 ng/0.5 mL of plasma.

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 5 mL of ethyl acetate. The solution was vortexed for 10 min to extract the drug into organic layer and then centrifuged at 3,000 rpm for 10 min. The organic layer was separated and dried by evaporation under vacuum. The residue was reconstituted with 0.3 mL methanol and filtered. The resulting filtrate was injected in to reverse phase C-18 column and the eluents monitored at 254 nm. The peak areas of the ornidazole were recorded and the regression of the plasma concentration over its peak area was calculated using the least square method of analysis.

Precision: Aliquots of blank plasma (500 μL) was spiked with 100 μL of ornidazole solution so as to yield concentrations of 20, 40 or 100 ng/0.5 mL. Each 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 injected into 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 ornidazole (50, 100 or 200 ng) and subjected to the proposed HPLC method, in triplicate. The difference in the measured concentration and that of the added quantity (50, 100 or 200 ng/0.5 mL) was expressed in terms of per cent recovery.

RESULTS AND DISCUSSION

The present study was carried out to develop a specific, sensitive, precise and accurate HPLC method for the analysis of ornidazole in human plasma samples. The run time of the method was set at 10 min. The retention time of ornidazole was 5.35 min (Fig. 1). When the same drug solution was injected 6 times, the

retention time of the drug was same. This indicates that the proposed HPLC method is rapid and accurate. Table-1 shows the mean peak area of ornidazole for 6 such determinations. When the concentration of ornidazole and its respective peak area was subjected to regression analysis by least squares method, a high correlation coefficient was observed (r = 0.99991 ± 0.046) in the range of 20 to 800 ng/0.5 mL only. However, the minimum quantifiable concentration was found to be 10 ng /0.5 mL of human plasma. The regression of ornidazole concentration over its peak area was found to be Y = 52.05 + 54.92X where 'Y' is the peak area and 'X' is the concentration of ornidazole. This regression equation is used to estimate the amount of ornidazole in plasma or in validation study (precision and accuracy).

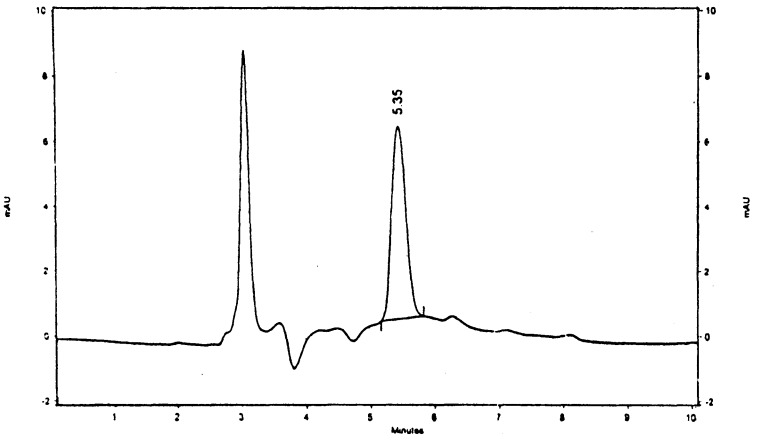

Fig. 1. Typical HPLC chromatogram of ornidazole in human plasma

TABLE- 1
CALIBRATION AND PRECISION OF THE HPLC ASSAY

Amount of ornidazole added to 0.5 mL human plasma (ng)	Peak area	%C.V.
10	542	0.00
20	1074	0.03
40	2620	0.10
100	5250	0.28
200	11654	0.03
400	22089	0.70
800	45108	0.20

*Mean of five determinations
Regression equation: Y= 52.05 + 54.95X (r = 0.9999)

The present HPLC method was also validated for intra- and inter-day variation. To asses the assay recovery from plasma by the present HPLC method, the plasma samples containing the drug (20, 40 and 100 ng/0.5 mL) were extracted as per the procedure described above, and the resultant filtrate samples were repeatedly injected on the same day and on three different days. The coefficient of variation (CV) in the peak area of ornidazole 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 1.6%. Thus the results show that the present HPLC method is highly reproducible. When a known amount of ornidazole (50 ng/mL) was added to preanalysed plasma samples (50, 100 or 200 ng/mL), there was a high recovery (99.83%) of the drug indicating that this HPLC method is highly accurate. The present HPLC method is found to be simple, precise and highly accurate for the estimation of ornidazole in human plasma.

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