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Development of a HPLC Method for the Estimation of Mebendazole in Human Plasma

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A HPLC method for the estimation of mebendazole in human plasma was developed. Varying amounts of mebendazole (5 to 400 ng), fixed quantity (0.5 μg) of tinidazole (internal standard) and 0.2 mL of 20% trichloroacetic acid solution were added to drug-free 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 filtered. Twenty microlitres of the resultant filtrate was injected into a reverse phase C-18 column using a mobile phase consisting of acetonitrile and water (containing 0.4% triethylamine and pH adjusted to 4.0 with 5% orthophosphoric acid) in the ratio of 38 : 62%v/v at a flow rate of 0.8 mL/min. The eluents were monitored at 254 nm. The method was validated for its linearity, precision and accuracy. The results of the study show that the present HPLC method is simple, precise, specific, less time consuming and accurate for the estimation of mebendazole in human plasma samples.

Key Words: Mebendazole, Estimation, Human plasma, HPLC.

INTRODUCTION

Mebendazole, chemically is methyl *n*-(5-benzoyl-1*h*-benzimidazol-2-yl) carbamate. It is highly effective against gastrointestinal nematode infections and is particularly valuable for the treatment of mixed infections. Mebendazole is the drug of choice for treating helminthicasis including trichuriasis (whipworm infections), ancylostomiasis (hookworm infections) and ascariasis[1]. Several analytical methods have been reported for the estimation of mebendazole or its metabolites in plasma by HPLC[2–6] and muscle tissue[7]. Some of the reported HPLC methods[5,6] required coulometric and electrochemical detectors and the process is considered tedious. 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 the estimation of mebendazole in human plasma using RP C-18 column and simple UV detection.

EXPERIMENTAL

Mebendazole was a gift sample from M/s CIPLA Ltd, Bangalore, India and tinidazole (used as internal standard) was a gift sample from M/s East India

Pharmaceutical Works Ltd, Kolkata, India. Triethylamine, orthophosphoric acid and trichloroacetic acid were of analytical grade and supplied by M/s SD Fine-Chem Ltd, Mumbai, India. Acetonitrile and water used were of HPLC grade (Qualigens).

Instrumentation: A gradient high pressure liquid chromatography (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) and RP C-18 column (250 mm × 4.6 mm I.D.; particle size 5 m; YMC, Inc., Wilmington, NC 28403, USA) 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 (containing 0.4% triethylamine and pH adjusted to 4.0 with 5% orthophosphoric acid) were filtered through 0.2 μm membrane filter before use and were pumped from the solvent reservoir in the ratio of 38 : 62% v/v to the column at a flow rate of 0.8 mL/min. The detector sensitivity was set at 0.0001 a.u.f.s. The volume of each injection was 20 μL.

Procedure: Seven 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 mebendazole at a concentration range of 5 to 400 ng/0.5 mL of plasma along with 100 μL of 0.5 g /0.5 mL of tinidazole (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 addition of 100 μL of 0.5 g/0.5 mL of tinidazole (internal standard) solution and 0.2 mL of 20% trichloroacetic acid. The mixture was vortexed to ensure complete mixing of contents for 5 min and centrifuged for 10 min at 3,000 rpm. The supernatant liquid was filtered through 0.2 μm membrane filter and twenty microlitres of the filtrate was injected into a reverse phase C-18 column and the eluents were monitored at 254 nm. The peak areas of mebendazole and internal standard (tinidazole) were recorded. The ratio of peak area of mebendazole to that of internal standard (tinidazole) was calculated, and the regression of the peak area ratio over the plasma concentration of the drug was calculated using the least squares method of analysis.

Precision: Aliquots of blank human plasma (0.5 mL) were spiked with 100 μL of internal standard solution (containing 0.5 μg/0.5 mL) and of mebendazole solutions (100 μL) so as to yield concentrations of 10, 50 and 100 ng/0.5 mL of plasma. Each sample was treated, as described above and the filtrate was 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 50 ng/ 0.5 mL were added with known quantity of mebendazole (10, 20 or 50 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 (10, 20 or 50 ng/0.5 mL) was expressed in terms of per cent recovery.

RESULTS AND DISCUSSION

The run time of the method was set at 12 min. Mebendazole and tinidazole (internal standard) appeared on the chromatogram at 9.8 min and 5.3 min respectively (Fig. 1). When the same drug solution was injected 5 times, the retention time of the drug and internal standard was the same. Table-1 shows the mean peak area ratios of mebendazole to that of internal standard solutions for 5 such determinations. When the concentration of mebendazole and its respective peak area ratios were subjected to regression analysis by least squares method, a high correlation coefficient was observed ($r = 0.9995 \pm 0.0361$) in the range of 10 to 400 ng/0.5 mL only. However, the minimum quantifiable concentration was found to be 5 ng/0.5 mL of human plasma. The regression of mebendazole concentration over its peak area ratio was found to be $Y = 0.00129 + 0.02503X$ where 'Y' is the peak area ratio and 'X' is the concentration of mebendazole. This regression equation was used to estimate the amount of mebendazole in plasma or in validation study (precision and accuracy).

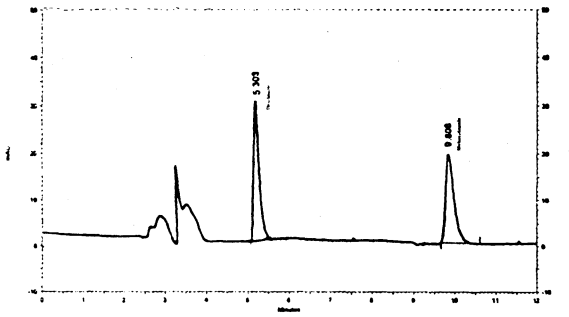

Fig. 1. Typical HPLC chromatogram of mebendazole in human plasma

TABLE -1
CALIBRATION CURVE FOR THE ESTIMATION OF MEBENDAZOLE IN HUMAN PLASMA BY HPLC METHOD

Amount of mebendazole added to 0.5 mL human plasma (ng)	Peak area *	C.V. (%)
0	0	0.00
5	0.0651	0.12
10	0.3018	0.39
20	0.6057	0.43
50	1.3315	0.80
100	2.3244	0.32
200	4.8762	0.21
400	10.1806	0.62

*Mean of seven determinations
Regression equation: $Y = 0.00129 + 0.02503X$ ($r = 0.9995$) in the range of 10 to 400 ng/0.5 mL of plasma.

The present HPLC method was also validated for intra- and inter-day variation. To assess the assay recovery from plasma by the present HPLC method, the drug (10, 50 and 100 ng/mL) was added to drug-free plasma along with internal standard solution. The plasma samples were treated as per the procedure described above and the resultant filtrate were 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 2%. Thus, the results show that this HPLC method is highly reproducible. When a known amount of drug solution (50 ng/mL) was added to preanalysed plasma samples (10, 20 or 50 ng/mL), there was a high recovery (99.9%) of mebendazole indicating that this HPLC method is highly accurate. Hence the proposed HPLC method was found to be simple, precise, highly accurate, specific and less time consuming than the reported methods[2-7].

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