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Development and Validation of a Reversed-Phase HPLC Method for the Analysis of Ornidazole in Pharmaceutical Dosage Forms

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The present study describes the estimation of ornidazole in pharmaceutical dosage forms. Ornidazole was chromatographed on a reverse phase C-18 column using mebendazole as an internal standard in a mobile phase consisting of acetonitrile and water (consisting of triethylamine and adjusted to pH 3.6 with 5% orthophosphoric acid) in the ratio of 38 : 62 v/v respectively. The mobile phase was pumped at a flow rate of 0.8 mL/min and the eluents were monitored at 254 nm. The method was statistically validated for its linearity, precision and accuracy. The calibration curve was linear in the range of 0.1 to 40 μg/mL. The intra- and inter-day variation was found to be less than 1% indicating high precision of the assay method. The mean recovery of the drug from the solutions containing 5, 10 or 20 μg/mL was 98.47 ± 0.37% indicating high accuracy of the proposed HPLC method. It does not require any specific sample preparation except the use of a guard column before the analytical column and a suitable pre-filter attached to the syringe prior to injection. Due to its simplicity, rapidness, high precision and accuracy, the proposed HPLC method may be used for the estimation of ornidazole in bulk drug samples or in pharmaceutical dosage forms.

Keywords: Ornidazole, Mebendazole, Reverse-phase HPLC, Pharmaceutical dosage forms.

INTRODUCTION

The objective of the present study is to develop a sensitive and specific method for the estimation of ornidazole in tablet dosage forms by HPLC using reverse phase RP C-18 column. A few analytical methods have been reported for the estimation of ornidazole in pharmaceutical dosage forms using UV spectrophotometry[1], electro-chemical reduction method[2] and HPLC[3]. The HPLC methods are considered simple, sensitive, precise, highly accurate, and require small quantity of sample. The HPLC method using the most commonly available columns and detectors like UV are preferred. The present study describes the determination of ornidazole in bulk drug samples and "pharmaceutical dosage forms" by using RP C-18 column with UV detection. Owing to the widespread

use of HPLC in routine analysis, it is important that well validated HPLC methods are to be developed for estimating ornidazole.

EXPERIMENTAL

Ornidazole (98–101% purity) and mebendazole gift samples from M/s Aristo Pharmaceuticals Limited, Bombay, India and M/s CIPLA, Bangalore, India respectively. Acetonitrile and water used were HPLC grade (Qualigens). All other reagents used in the study such as triethylamine and orthophosphoric acid 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^{TM}, LC-18, 2 cm, Supelco, Inc., Bellefonte, PA.) and RP C-18 column (250 mm × 4.6 mm I.D., particle size 5 m; YMC Inc., USA) was used. The HPLC system was equipped with the software "Class-VP series version 5.03 (Shimadzu)".

HPLC conditions: The contents of the mobile phase, acetonitrile and water (consisting of 0.4% triethylamine and pH adjusted to 3.6 with 5% orthophosphoric acid) in the ratio of 38 : 62 v/v, were filtered before use through 0.2 μm membrane filter and degassed with a helium spurge for 15 min. The components of the mobile phase were pumped from the respective solvent reservoirs to the column at a flow rate of 0.8 mL/min, which yielded a column back-pressure of 120–130 kg/cm^2. The run time was set at 10 min and the column temperature was maintained at 40°C. The volume of the injection loop was 20 μL. The eluents were monitored with UV detection at 254 nm.

Procedure: Stock solution of ornidazole and internal standard (mebendazole) was prepared by dissolving 100 mg of ornidazole and mebendazole in 100 mL volumetric flasks containing 70 mL of methanol and acetic acid respectively, sonicated for 10 min and then volume made to 100 mL so as to give 1 mg/mL solution. Daily working standard solutions of ornidazole and mebendazole were prepared by suitable dilution of the stock solution with mobile phase.

Six sets of the ornidazole solution were prepared in methanol at concentrations of 0.1, 0.2, 0.5, 1, 2, 4, 8, 10, 20 and 40 μg/mL along with a fixed quantity (2 μg) of mebendazole as internal standard. Each of these samples (20 μL) was injected six times into the column and the peak area of the drug and internal standard were recorded.

Assay of ornidazole in tablets: Twenty tablets were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 100 mg of ornidazole was placed in a 100-mL volumetric flask. Seventy millilitres of methanol were added and the flask was allowed to stand for 5 h with intermittent sonication to ensure complete solubility of the drug. The mixture was then made up to 100 mL with methanol, thoroughly mixed and filtered through a 0.2 μm membrane filter. An aliquot of this filtrate (2 mL) was transferred to a volumetric flask along with appropriate volume of mebendazole solution and made up to

volume with methanol to give an expected concentration 10 μg/mL of ornidazole and 2 μg/mL of mebendazole (internal standard). All determinations were conducted in triplicate. The same procedure was used to estimate the concentration of the drug in two more commercial brands of ornidazole tablets.

Validation: The proposed HPLC method was validated in terms of linearity, precision and accuracy. The precision (% coefficient of variation) was expressed with respect the inter- and intra-day variation in the expected drug concentration. The accuracy was expressed in terms of per cent recovery of known amount of drug added to the known amount of preanalysed ornidazole solution.

Linearity: The linearity of the proposed HPLC method was determined in terms of the correlation coefficient between the concentration of the drug and its respective peak area ratio to that of internal standard. The data were subjected to regression analysis using least square method.

Precision: The precision of the assay was determined in terms of intra- and inter-day variation in the peak area ratio for a set of drug solutions on three different days (n = 5). The intra- and inter-day variation in the peak area ratio of the drug solution to that of internal standard was calculated in terms of coefficient of variation (C.V.), and was obtained by multiplying the ratio of standard deviation to the mean with 100 [C.V = (SD/mean) × 100].

Accuracy: The accuracy of the HPLC assay method was assessed by adding known amount (5, 10 or 20 μg/mL) of the drug to a drug solution of known concentration along with 2 μg/mL internal standard and subjecting the samples to the proposed HPLC method. Also, known amount of drug solution (10 or 20 μg/mL) was added to the volumetric flask containing the powdered sample of the tablet formulation with known amount of the drug and internal standard. The drug was estimated as per the procedure described above for the estimation of ornidazole in tablet formulations. In both the cases, the recovery studies were replicated five times. The accuracy was expressed in terms of the recovery and was calculated by multiplying the ratio of measured drug concentration to the expected drug concentration with 100 so as to give the per cent recovery.

RESULTS AND DISCUSSION

The development of an analytical method for the determination of drugs by HPLC has received considerable attention in recent years because of their importance in quality control of drugs and drug products. The goal of this study was to develop a rapid HPLC method for the analysis of ornidazole in tablet formulations using the most commonly employed RP C-18 column with UV detection.

The run time of the method was set at 10 min. The retention times of ornidazole and mebendazole (internal standard) were 3.70 min and 7.65 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 present 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 ornidazole solutions for 6 such determinations. When the concentration of

ornidazole and its respective peak area ratios were subjected to regression analysis by least squares method, a high correlation coefficient was observed ($r = 0.9999 \pm 0.058$) in the range of 0.1 to 40 μg/mL. The regression of ornidazole concentration over its peak area ratio was found to be $Y = -0.00057 + 0.02556X$ where 'Y' is the peak area ratio and 'X' is the concentration of ornidazole. This regression equation was used to estimate the amount of ornidazole either in tablet formulations or in validation study (precision and accuracy).

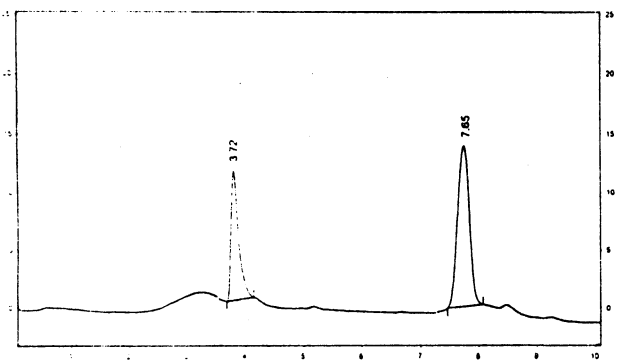

Fig. 1. A typical chromatogram of ornidazole

TABLE- 1
CALIBRATION AND PRECISION OF THE HPLC ASSAY

Concentration of ornidazole (μg/mL)	Peak area ratio*	C.V. (%)
0.0	0	0.00
0.1	0.0001	0.50
0.2	0.0051	0.86
0.5	0.0135	1.40
1.0	0.0265	0.89
2.0	0.0579	0.55
4.0	0.1108	0.59
8.0	0.2201	1.31
10.0	0.2641	0.20
20.0	0.5242	2.11
40.0	1.0452	1.18

*Mean of six determinations
Regression equation: $Y = -0.00057 + 0.02556X$ ($r = 0.99987$)

The proposed HPLC method was also validated for intra- and inter-day variation. When the solutions containing 5, 20 or 40 μg/mL of ornidazole along with 2 μg/mL of mebendazole were repeatedly injected on the same day, the coefficient of

variation (CV) in the peak area ratio of the drug for five replicate injections was found to be less than 1%. Also, the inter-day variation (3 days and five injections) was found to be less than 1% (Table-2). Thus, the results show that the proposed HPLC method is highly reproducible. When a known amount of drug solution (10 or 20 μg/mL) was added to a preanalysed samples of drug solution (20 μg/mL), there was a high recovery (98.47 ± 0.37%) of ornidazole indicating that the proposed HPLC method is highly accurate.

TABLE- 2
INTER- AND INTRA-DAY PRECISION FOR ORNIDAZOLE ASSAY IN PHARMACEUTICAL DOSAGE FORMS BY THE PROPOSED HPLC METHOD

Actual concentration of ornidazole (μg/mL)	Observed concentration of ornidazole (μg/mL)			
	(Intra-day)		(Inter-day)	
	Mean*	% CV	Mean*	% CV
5	5.01	0.71	4.99	0.89
20	20.03	0.65	20.01	0.47
50	40.02	0.53	40.01	0.36

* Mean of five determinations

The HPLC method, developed in the present study, has also been used to quantify ornidazole in tablet dosage forms. Ornidazole tablets (containing 500 mg of the drug) were analyzed and the results obtained were given in Table-3. The average drug content was found to be 99.78% of the labeled amount. No interfering peaks were found in the chromatogram indicating that the tablet excipients did not interfere with the estimation of the drug by the proposed HPLC method. Also, when a known amount of the drug solution was added to the powdered sample of the tablet dosage form and subjected to the estimation of the drug by the proposed method, there was high recovery of ornidazole (99.47 ± 0.31%) indicating that the proposed procedure for the determination of ornidazole in the tablet dosage forms is highly accurate.

TABLE -3
MEAN (S.D) AMOUNT OF ORNIDAZOLE IN TABLET DOSAGE FORMS BY PROPOSED HPLC METHOD

Brand of the tablet (n = 3)	Labeled amount (mg)	Observed amount (mg)	Purity (%)
AA	500	497.49	99.45 ± 1.1
BB	500	498.93	99.78 ± 1.7
CC	500	498.02	99.60 ± 0.8

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