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Research Article

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Quantification of Ornidazole from bulk and pharmaceutical dosage form using a validated chromatographic technique

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ABSTRACT

The current study presents a simple, rapid, precise technique for the quantification of Ornidazole from bulk and pharmaceutical formulations. The analysis was carried out using a C18 column (250 mm × 4.6 mm; 5 µm) using a combination of 0.53 mM phosphate buffer and acetonitrile in mobile phase at a flow rate of 1ml per minute. The chromatogram presents curves free from co-elution at a detection wavelength of 305nm. The method has been validated as per ICH Q2 guidelines. The method was found to linear within concentration range 10.49µg/ml and 83.96µg/ml with limit of detection and limit of quantitation 0.17µg/ml and 0.52µg/ml respectively. The method was found to be suitable for the regular analysis of ornidazole form marketed formulations.

Keywords: Liquid Chromatography; Ornidazole; Validation.

INTRODUCTION

Ornidazole (ORND) is a nitro imidazole derivative (1-chloro-3-(2-methyl-5-nitro-1*H*-imidazol-1-yl) propan-2-ol) found to have antibacterial and antiprotozoal activity (Fig. 1). In the human body it is converted into its active form through reduction of the nitro group present that binds with the bacterial DNA and prevents nuclic acid formation elucidating its bacteriostatic properties [1, 2]. ORND find application in the treatment of bacterial vaginosis, trichomoniasis, genitourinary infections in both sexes due to *Trichomonas vaginalis*, amoebiasis due *Entamoeba histolytica* , amoebic dysentery, amoebic liver abscess and giardiasis [3-7], anaerobic bacterial infections and post operative wound infections. It is also prescribed in a prophylacsis in colonic and gynaecological surgeries and to prevent post operative recurrences [8] in patients with Crohn's Disease and Inflammatory Bowel Disease [9]. Several formulations containing ORND as the only active ingredient in oral solid and liquid formulations are available in the market and are prescribed for the treatment of bacterial and protozoan infections.

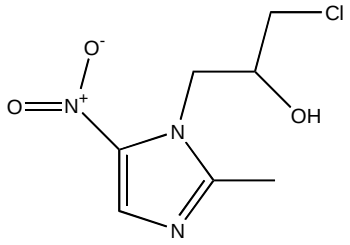

Fig. 1 Structure of Ornidazole

Several literature reports are present describing the quantitative analysis of ORND from bulk and tablet dosage forms. It is official in IP [10] which presents a potentiometric method for bulk drug and a UV spectrophotometric

method for formulations. Most of the methods presented in literature reports the application of spectrophotometry [11-16], liquid chromatography – HPLC [17-20] and HPTLC [21, 22], and voltametric [23] techniques for the quantification of ORND alone or in combination with other drugs. However, most of these methods are more complicated and time consuming and very few present the quantification of ORND alone from such formulations. The current study presents a simple, rapid, precise and accurate chromatographic technique for the quantification of ORND from bulk and formulations.

EXPERIMENTAL SECTION

Chemicals and reagents:

Standard Ornidazole was procured from Endoc Pharma, Gujarat, India as gift sample and was used as working standard without further purification. The pharmaceutical dosage form used in the study was Ornida (Aristo Pharmaceutical Ltd.) purchased from local market. HPLC grade acetonitrile, phosphoric acid and AR grade monobasic and dibasic potassium phosphate were purchased from Merck India Ltd., Mumbai, India. Water used for the preparation of all solution was of HPLC grade which was obtained from Aurium 611 UV purification system of Sartorius, Germany.

Instrumentation and chromatographic conditions:

High performance liquid chromatograph (Waters eAlliance e2695, Waters; USA) and 2489 dual lambda absorbance detector (Waters, USA) was used in this study [24-26]. Chromatographic separation was performed isocratically at room temperature using a reverse phase C_{18} column (250 mm × 4 mm, 5 µm particle size). The optimized mobile phase used in this study was a mixture of 5.3 mM phosphate buffer solution adjusted to pH 3.5 ± 0.1 with orthophosphoric acid and acetonitrile in the ratio of 60:40 %v/v. Flow rate was maintained at 1 ml/min run time for 5 min. The injection volume was 20 µl. The response was measured at 305 nm at ambient temperature. All data were analysed using Empower-3 software (Waters, USA).

Preparation of standard solution:

25 mg of ornidazole working standard was weighed accurately and transferred into a 25 ml clean and dry volumetric flask, 10 ml HPLC grade water and few drops of diluted hydrochloric acid were added to it followed by sonication for 10 minutes to dissolve completely and made the volume up to the mark with mobile phase. This stock solution was suitably diluted to obtain standard solutions in the concentration range 10.49µg/ml and 83.96µg/ml in mobile phase. The standard solution was filtered through 0.45 µm syringe filter before injection.

Preparation of sample stock solution:

20 tablets of ORND were accurately weighed and powdered using a mortar and pestle. An appropriate amount of powdered mass equivalent to 25 mg of ORND was weighed accurately and transferred into a 25 ml volumetric flask. To this, about 10 ml of HPLC grade water and few drop of diluted hydrochloric acid was added and sonicated for 10 minutes and the volume was made up with mobile phase. This solution was filtered through Whatman filter paper no. 1 and further diluted to 40 µg/ml with mobile phase. The sample solution was filtered through 0.45 µm syringe filter before injection.

Assay of the Commercial Sample:

10 µl of each standard solutions and sample solution were injected separately into the pre-equilibrated chromatographic system. Chromatograms of standard solutions (six replicates) and sample solution (three replicates) were recorded. A typical chromatogram of ORND was presented in Fig. 2. The drug content was calculated by comparing area of the sample solution with that of the standard solution. Results were presented in Table 1.

Table 1: Sample formulation

Formulation	Drug	Amount of Drug (mg/tab)		% of Label Claim	% RSD
		Labelled	Estimated*		
Ornida (Aristo Pharmaceutical Ltd.) 500 mg/Tab	ORND	500	499.98	99.99	0.21

** Mean from three replicate analyses.*

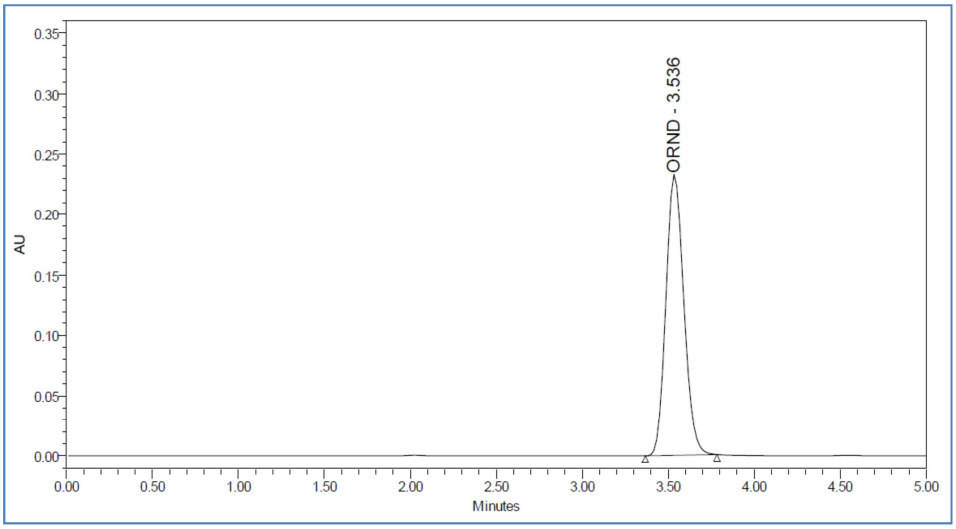

Fig. 2 Chromatogram of Ornidazole (retention time = 3.536)

METHOD VALIDATION

The proposed method was validated to meet the acceptance criteria of the USP [27] and ICH Q2 [28] guidelines for selectivity, system suitability, linearity, precision, accuracy, sensitivity, robustness and ruggedness.

Selectivity:

Selectivity of the method was determined by analyzing blank (mobile phase), to demonstrate the lack of chromatographic interference at the retention time of the analyte.

System suitability:

To ascertain resolution and reproducibility of the proposed HPLC method for estimation of ORND in formulation, system suitability parameters were studied. For this six replicate injections of the standard preparation were made in the chromatographic system and parameters such as column efficiency, resolution, peak asymmetry, retention time, tailing factor, theoretical plates have been determined (Table 2).

Table 2: System suitability parameter

Parameters	Ornidazole
Wavelength maxima (nm)	305
Retention time (mins)	3.536
Tailing factor	0.2946
Theoretical plate	222269
LOD (µg/ml)	0.17
LOQ (µg/ml)	0.52

Linearity:

Linearity was determined by taking five different concentrations of ORND in triplicate and calibration curves were plotted in the concentration range 10.49µg/ml and 83.96µg/ml (Fig. 3). The linearity was evaluated by linear regression analysis, which was calculated by least square method. The linear regression co-efficient was found to be 0.998 (Table 3).

Table 3: Linearity parameters

Parameters	Ornidazole
Linearity range (µg/ml)	10.49- 83.96
Regression coefficient	0.998
Intercept	2138
Slope	40680

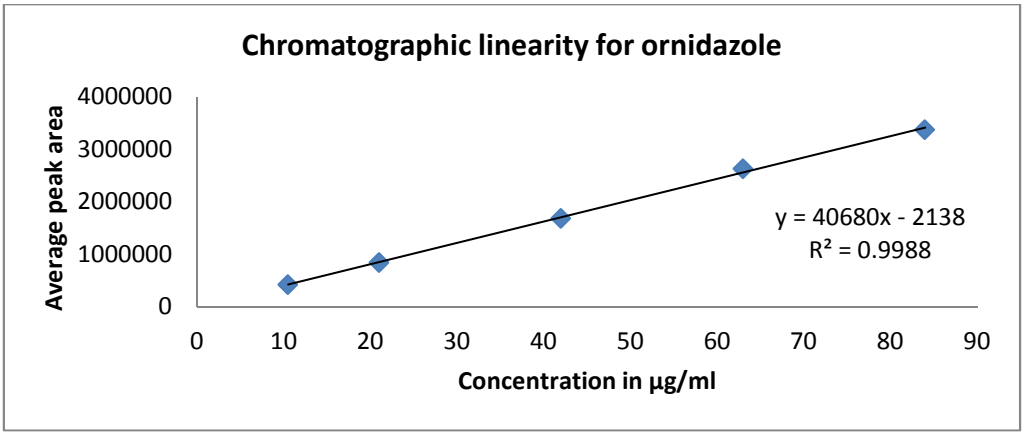

Fig. 3 Peak area verses concentration curve for Ornidazole

Precession:

Precession of the proposed analytical method which was the degree of agreement among individual test results was ascertained by intra-day and inter-day variation studies. The experiments were repeated three times a day for intra-day precision and three consecutive days for inter-day precision. The %RSD with respect to the peak area, peak retention time and the amount were calculated for each case and the results were deputed in Table 4.

Table 4: Precision parameters

Parameters	Intra-day	% RSD	Inter-day			
			Day1	Day2	Day3	% RSD
Peak Area	1686595	0.04	1686119	1686201	1685899	0.01
Peak RT	3.536	0.11	3.531	3.521	3.527	0.14
Amount (mg/Tab)	499.99	0.04	499.84	499.87	499.78	0.01

Accuracy:

Accuracy of the method i.e. closeness of the result obtained to the true value was determined in terms of percentage recovery. Sample solutions were prepared at three different concentration levels 80%, 110% and 120%. Predetermined amount of standard was added to three solutions by spiking standard drug solution to the sample. The percentage recovery and standard deviation of the percentage recovery were calculated and presented in Table 5.

Table 5: Accuracy parameters (recovery study)

Formulation	Drug	Labeled Amt. (mg/tab)	Assay amount (mg/tab)	% label claim (n =3)	Recovery Studies (n = 3)				
					Total Amt. after spiking (mg)	Amt recovered (mg) Mean ± SD	% Recovery	% Mean Recover	% RSD
Ornida (Aristo Pharmaceutical Ltd.)	Ornidazole	500.00	499.99	99.99	400 550 600	399.99±1.23 549.56±2.30 601.08±1.99	99.99 99.92 100.18	100.03	0.13

Sensitivity:

Sensitivity of the method was determined by calculating LOD (limit of detection) and LOQ (limit of quantification) using the equation: LOD = 3.3 σ/s and 10 σ/s where 'σ' was the standard deviation of response (y intercept) and's' was the slope of the calibration curve. The results were found to be 0.17µg/ml and 0.52µg/ml, respectively (Table 2).

Robustness and Ruggedness:

Robustness of the proposed method was satisfactorily determined by evaluating chromatographic characteristics at the small variation in method parameters like mobile phase composition (±2% organic phase), flow rate (± 0.02 ml/min) and pH (±5%).

Ruggedness of the method was done by studying changes with variation of analyst to analyst, column to column, instrument to instrument and provides an indication of its reliability.

RESULTS AND DISCUSSION

The aim of the present work was to develop method for the determination of ornidazole and validate the method according to USP [27] and ICH Q2 [28] guidelines and applying the same for its estimation in pharmaceutical formulations. Initially, various mobile phase composition was tried but based on peak parameters like area, height, capacity, theoretical plates, tailing factor, run time and resolution the best mobile phase was selected [29, 30]and it was a mixture of 5.3 mM phosphate buffer solution (pH 3.5±0.1) and acetonitrile in the ratio 60:40 %v/v. By using this mobile phase a satisfactory separation and good peak symmetry was obtained with a C_{18} column (250 mm × 4 mm, 5 µm) at a flow rate of 1 ml/min. Quantification was done with UV detection at 305 nm and the retention time was found to be 3.536 min (Fig.2). The system suitability parameters were calculated and were found within limits (Table 2). Linearity was evaluated in the concentration range of 10.49 – 83.96µg/ml. The calibration curve (Fig. 3) was described by the equation Y = 40680X - 2138 with regression co-efficient 0.998 as shown in Table 3. The low %RSD value of intraday and interday precision studies (Table 4) revealed high degree of precision of the proposed method. The results of formulation analysis and recovery studies (Table 5) were validated statistically indicating high degree of accuracy. LOD and LOQ were separately determined based on standard deviation of response of calibration curve. The values were 0.17µg/ml and 0.52µg/ml respectively (Table 2). The results of robustness study indicated that the method was robust and was unaffected by small variation in chromatographic condition. The method was satisfactory with respect to ruggedness also.

CONCLUSION

From all results, it can be concluded that the proposed RP-HPLC method was found to be simple, accurate, precise, rapid and useful for routine analysis of ornidazole in bulk and its pharmaceutical dosage form.

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