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Original Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ORNIDAZOLE IN TABLET DOSAGE FORM BY ZERO ORDER UV SPECTROSCOPY

JYOTSNA PATIL[1], MALATHI RAGHUNATH[2]*, SHWETA PATIL[3]

Department of Pharmaceutical and Medicinal Chemistry, Gahlot Institute of Pharmacy, Plot no. 59, Sector-14, Koparkhairane, Navi Mumbai 400709, Maharashtra, India
Email: malathiraghu12@gmail.com

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ABSTRACT

Objective: To develop and validate two simple and cost effective spectrophotometric methods for the determination of Ornidazole (OND) in pure form and in pharmaceutical tablet dosage form.

Methods: Single point standardization method was used for quantitative estimation of OND. In method 1 absorbance was measured at 311 nm (λ_{max} of OND in methanol) using methanol as a solvent system and in method 2 absorbance was measured at 277 nm (λ_{max} of OND in 0.1 N HCl) using 0.1 N HCl as the solvent system.

Results: The two methods obeyed Beer's law in the concentration range of 3-18 µg/ml (methanol) and 10-35 µg/ml (0.1 N HCl) respectively. The methods were validated for parameters such as linearity, accuracy, and precision, limit of detection (LOD), limit of quantification (LOQ), robustness and ruggedness as per ICH guidelines.

Conclusion: The developed methods were used for determining the content of OND in commercial tablets. The proposed methods were thus found to be suitable for quantitative estimation of OND in bulk and tablet formulation without any interference of the excipients and can be employed for quality control of Ornidazole in tablet dosage form.

Keywords: Ornidazole, Validation, Single point standardization, ICH guidelines

INTRODUCTION

Ornidazole (OND), a 5-nitroimidazole is used in the treatment of protozoal infections and also in the treatment and prophylaxis of anaerobic infections. It has been investigated for use in Crohn's disease after bowel resection. OND is converted into reduction products that interact with DNA to cause the destruction of the helical DNA structure and strand leading to inhibition of protein synthesis causing cell death in susceptible organisms. Chemically, it is 1-chloro-3-(2-methyl-5-nitro-1 H-imidazol-1-yl propan-2-ol and its structure is shown in fig. 1. It is used either as monotherapy or in combination with cephalosporins and in fluoroquinolone antibiotics [1-6]. OND is not official in any Pharmacopoeia.

The methods that have already been reported for estimation of OND in bulk and in the formulation are UV-Spectrophotometry, RP-HPLC and HPTLC. UV-Spectrophotometric methods have been reported using ethanol and distilled water as a solvent for estimation of OND alone [7, 8]. OND in its various drug combinations have been estimated using UV-Spectrophotometry [9-16], RP-HPLC [17, 18] and HPTLC [18, 19]. To the best of our knowledge, the quantitative estimation of OND alone in methanol and 0.1 N HCl by zero order UV-Spectrophotometry has not been reported yet. Thus, an attempt was made to develop two simple, accurate and precise zero order UV-Spectroscopic methods for determination of OND using methanol and 0.1 N HCl as the solvent system at its respective λ_{max} values.

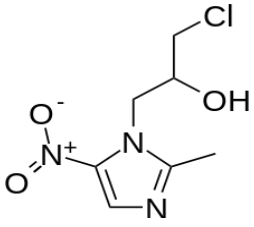

Fig. 1: It shows chemical structure of Ornidazole [3]

MATERIALS AND METHODS

Spectrophotometric measurements were done using a double beam LABINDIA 3000+UV/Visible spectrophotometer and a pair of 1 cm matched quartz cells was used. All samples were weighed on Shimadzu digital balance (Model No. AUY220). All chemicals and reagents were of analytical grade. OND was obtained as a gift sample from Nicholas Piramal, Ltd; Goregaon, Mumbai. It was authenticated before use by performing identification tests. Owing to the good solubility of OND in methanol and 0.1 N HCl, these two solvents were chosen to dissolve the drugs.

Preparation of stock solution

Method 1

A quantity of 25 mg of OND was accurately weighed and dissolved in 25 ml methanol to get a solution containing 1 mg/ml of OND.

Method 2

Similarly, a stock solution having a concentration of OND as 1 mg/ml was prepared by dissolving 25 mg drug in 0.1 N HCl and making up the volume to 25 ml with the same solvent.

Preparation of linearity solutions

The stock solutions of OND in methanol and 0.1 N HCl were serially diluted to obtain solutions ranging from 3-18 µg/ml (methanol) and 10-35 µg/ml (0.1 N HCl) respectively.

Determination of wavelength

A solution of a suitable concentration of OND in methanol and 0.1 N HCl was scanned in UV range from 200-400 nm to determine the wavelength of maximum absorption. For linearity determination, validation studies and assay of OND in the marketed formulation, absorbance measurements were carried out at 311 nm and 277 nm using methanol and 0.1 N HCl respectively. The zero order UV

spectrum of OND in methanol and 0.1 N HCl was recorded. In order to confirm the λ_{max}, first order spectrum was recorded for both the methods. The zero crossing points were observed at 262 and 311 nm (methanol) and 241 and 277 nm (0.1 N HCl).

Calibration curve

Method 1

From the stock solution of OND (1 mg/ml), 1 ml was pipetted out and transferred to 10 ml volumetric flask. The volume was made up with methanol. From this solution (100 µg/ml), various linearity solutions were prepared ranging from 3-18 µg/ml by pipetting out 0.3, 0.6, 0.9, 1.2, 1.5, 1.8 ml respectively into 10 ml volumetric flasks and volume was made up to 10 ml using the same solvent.

Method 2

Similarly from the stock solution of OND (1 mg/ml), 5 ml was pipetted out and transferred to 50 ml volumetric flask. The volume was made up with 0.1 N HCl. From this solution (100 µg/ml), various linearity solutions were prepared ranging from 10-35 µg/ml by pipetting out 1, 1.5, 2, 2.5, 3, 3.5 ml respectively into 10 ml volumetric flasks and volume was made using the same solvent.

The calibration curves were generated. The zero order and first order spectra of linearity solutions for the two proposed methods were recorded.

Assay of Ornidazole in bulk and in tablet dosage form

The assay of Ornidazole in bulk was carried out by accurately weighing and transferring a quantity of 25 mg of the drug to two separate 25 ml volumetric flask and making up the volume with methanol and 0.1 N HCl. The solutions were suitably diluted, and their absorbance was recorded at 311 nm (λ_{max} of OND in methanol) and 277 nm (λ_{max} of OND in 0.1 N HCl).

The assay of OND tablets (ORNI 500; manufactured by Zydus Healthcare, Sikkim) was carried out by determining the average weight of 20 tablets. The tablets were then ground to fine powder and mixed thoroughly. A quantity of 33 mg of tablet powder equivalent to 25 mg of OND was accurately weighed and transferred to two separate 25 ml volumetric flasks. After adding the respective solvents, the solutions were sonicated for 20 min to extract the drug completely in methanol and 0.1 N HCl. The volume was made up with the same solvents. The solutions were filtered through Whatman filter paper no. 41 and were suitably diluted. The absorbance of solutions was measured at their respective λ_{max} values against the solvent as blank.

Method validation

The proposed methods were evaluated for various validation parameters as per ICH guidelines [20]. Intra-day and inter-day precision were determined at three different time intervals on same and different days by analyzing the drug solution having concentration 15 µg/ml (methanol) and concentration 20 µg/ml (0.1 N HCl). The % RSD was calculated. The accuracy of the method was evaluated by carrying out recovery experiments at three levels, and percentage recovery was determined. Limit of detection (LOD) and Limit of quantification (LOQ) was calculated from calibration curves using the respective slope and standard deviation of y-intercepts.

RESULTS

The UV scans of OND in methanol and 0.1 N HCl are shown in fig. 2 and 3 respectively. Similarly, the first order spectra of both the methods are shown in fig. 4 and 5. The calibration curves are shown in fig. 6 and 7. Zero and first order overlay spectra of linearity solutions of OND were constructed in methanol and were shown in fig. 8 and 9 respectively. Similarly, fig. 10 and 11 show zero and first order spectra of linearity solutions of OND in 0.1 N HCl respectively. The results of regression analysis for quantification of OND in methanol and 0.1 N HCl is shown in table 1.

The accuracy and recovery studies were carried out on tablet formulation for both the methods and the results are shown in table 2 and 3 respectively. The results of reproducibility, intra-day precision and inter-day precision are shown in table 4, 5 and 6 respectively. Robustness of the methods was determined by carrying out the analysis under different operating conditions such as wavelength and variation in concentrations. The respective absorbance was noted, and the result was expressed as % RSD as shown in table 7. The ruggedness of the methods was also determined by carrying out the analysis by different analyst and the respective absorbance of the solution of OND having concentration as 15µg/ml for method 1 and 20µg/ml for method 2 were noted. The results are shown in table 8. The result of the assay of OND on the tablet dosage form is shown in table 9.

Table 1: It shows regression analysis of Ornidazole in methanol and 0.1 N HCl

Parameters	Method 1	Method 2
Solvent	Methanol	0.1 N HCl
Linearity range (µg/ml)	3-18 µg/ml	10-35 µg/ml
Wavelength, λ_{max} (nm)	311	277
Regression equation	y=0.035x+0.001	y=0.028x+0.010
Correlation coefficient (r^2)	0.999	0.999
Molar absorptivity, ε (L/mol/cm)	8873	6391
Sandell's sensitivity (µg. cm^2/0.001 absorbance unit)	0.025	0.034
y-intercept	0.001	0.010
95% confidence interval for slope	1.25354×10^{-5}	1.25354×10^{-5}
95% confidence interval for intercept	7.57731×10^{-5}	1.9889×10^{-4}
LOD (µg/ml)	0.019	0.84
LOQ (µg/ml)	0.041	2.53

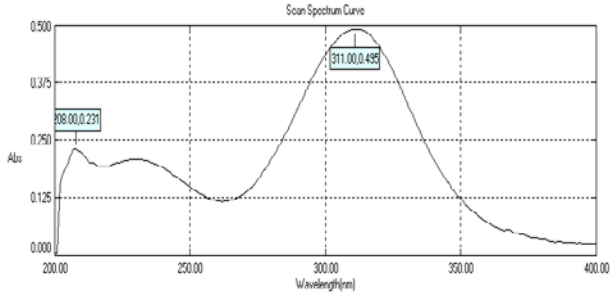

Fig. 2: It shows UV spectrum of Ornidazole in methanol

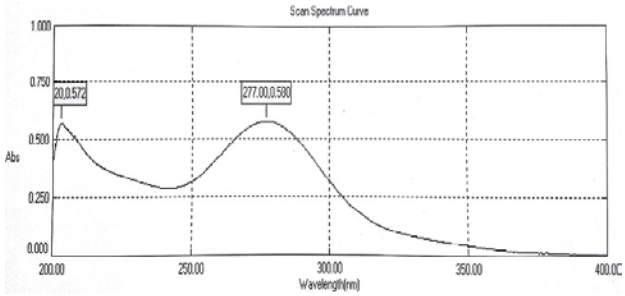

Fig. 3: It shows UV spectrum of Ornidazole in 0.1 N HCL

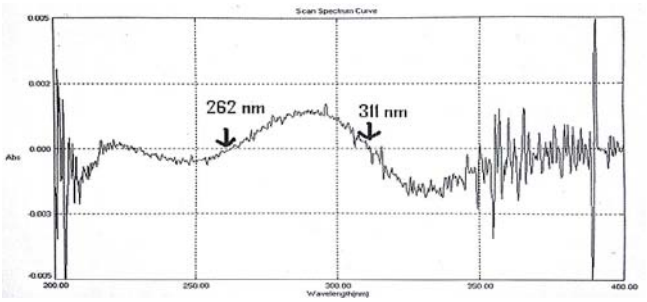

Fig. 4: It shows first order spectrum of OND in methanol

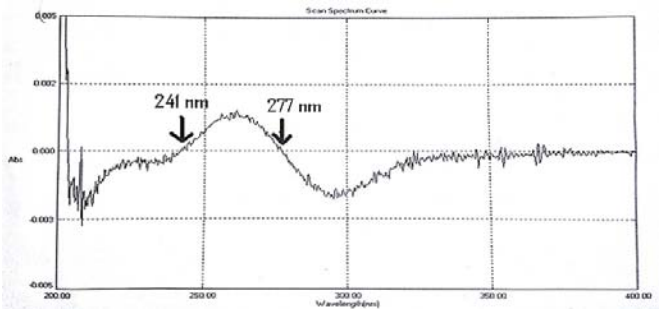

Fig. 5: It shows first order spectrum of OND in 0.1 N HCl

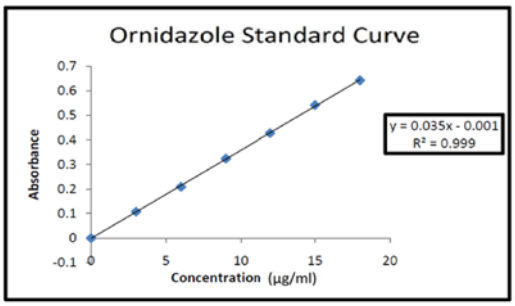

Fig. 6: It shows calibration curve of Ornidazole in methanol

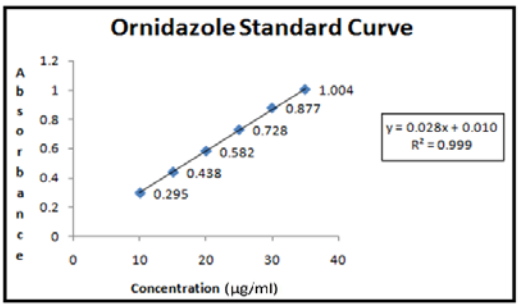

Fig. 7: It shows calibration curve of Ornidazole in 0.1 N HCl

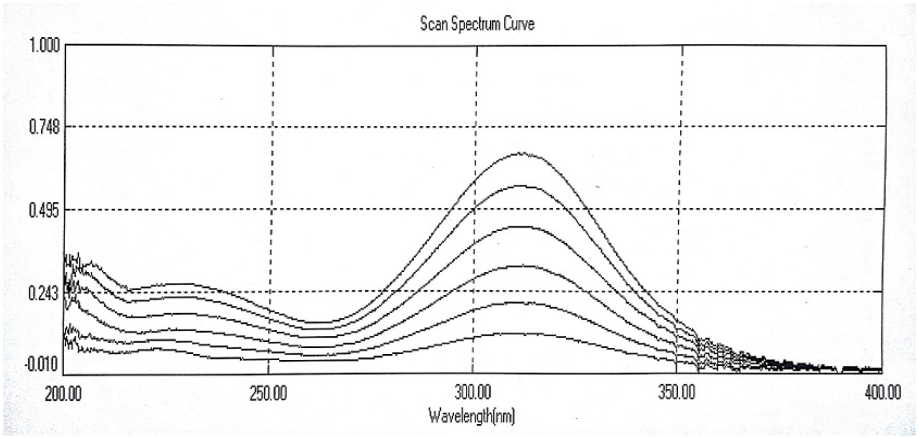

Fig. 8: It shows overlain zero-order spectrum of linearity solutions of OND in methanol

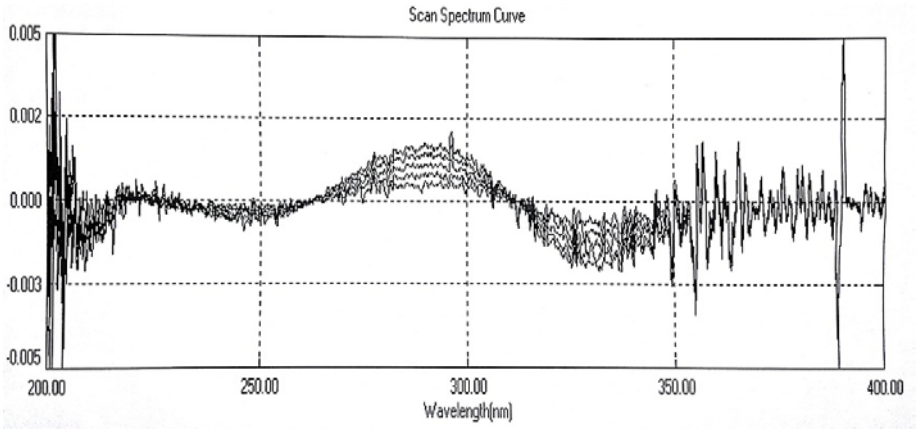

Fig. 9: It shows overlain first order spectrum of linearity solutions of OND in methanol

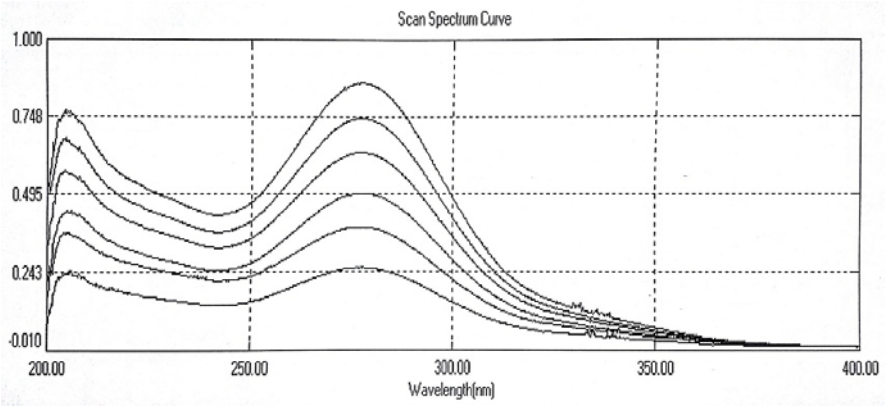

Fig. 10: It shows overlain zero-order spectrum of linearity solutions of OND in 0.1N HCl

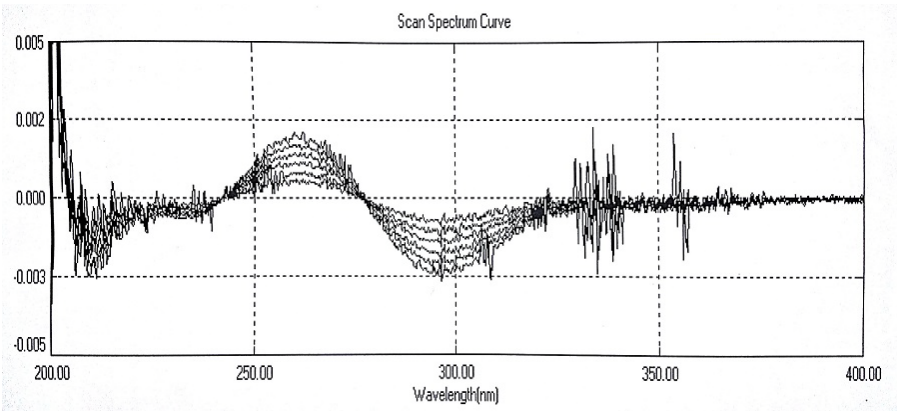

Fig. 11: It shows overlain first order spectrum of linearity solutions of OND in 0.1 N HCl

Table 2: It shows results of accuracy studies for method 1 and 2

Recovery level		Sample concentration (µg/ml)	% Recovery* (average of three determinations±SD)	%RSD	SE
Method 1	80%	12	100.6±0.7286	0.725	0.420
	100%	15	103.2±0.380	0.370	0.220
	120%	18	101.8±0.410	0.403	0.237
Method 2	80%	16	98.2±1.175	1.196	0.680
	100%	20	98.6±1.013	1.028	0.585
	120%	24	99.4±1.379	1.390	0.796

t-calculated value: 0.01034; F-calculated value: 0.910923, t-tabulated value (P = 0.05): 2.132; F-tabulated value (P = 0.05, df_1= 2, df_2= 2): 19.00, *mean of three determinations at each level, P= level of significance, SD= Standard deviation (n= 3), RSD= Relative standard deviation, SE= Standard error (n= 3), df_1 and df_2= degrees of freedom for method 1 and method 2 respectively.

Table 3: It shows results of Recovery studies of Ornidazole in marketed formulation for method 1 and 2

Tablet formulation	Recovery level (%)	Amount of drug in tablet powder (mg)	Amount of drug-spiked (mg)	% Recovery (average of three determinations±SD)	% RSD	SE
Method 1	80	25	16	100.73±1.545	1.530	0.890
	100	25	20	101.75±1.190	1.170	0.690
	120	25	24	100.01±1.169	0.220	0.645
Method 2	80	25	16	99.91±0.336	0.337	0.190
	100	25	20	96.12±0.095	0.099	0.055
	120	25	24	97.9±0.129	0.132	0.074

Table 4: It shows results of reproducibility studies for method 1 and 2

Observations	Method 1	Method 2
Concentration	15µg/ml	20µg/ml
Wavelength	311 nm	277 nm
Absorbance	0.657	0.563
Mean (average of 9 determinations)	0.670	0.559
SD	0.014	0.004
%RSD	0.521	0.747

Table 5: It shows results of intra-day precision for method 1 and 2

Time	%RSD	
	Method 1	Method 2
10.00 am	0.463	0.330
1.00 pm	0.532	0.665
4.00 pm	0.611	0.480
Average %RSD	0.535	0.490

Table 6: It shows results of inter-day precision for method 1 and 2

Day	%RSD	
	Method 1	Method 2
Day 1	0.571	0.890
Day 2	0.655	0.787
Day 3	0.460	1.360
Average %RSD	0.562	1.011

Table 7: It shows results of robustness for method 1 and 2

Method	Wavelength (nm)	Concentration (µg/ml)	Absorbance (mean of three determinations) (n = 3)	% RSD
Method 1	309	12	0.428	0.467
		15	0.539	0.283
		18	0.642	0.412
	311	12	0.431	0.837
		15	0.542	0.384
		18	0.644	0.237
	313	12	0.428	0.713
		15	0.537	0.468
		18	0.642	0.311
Method 2	275	16	0.455	1.249
		20	0.564	0.569
		24	0.686	1.27
	277	16	0.458	1.240
		20	0.568	0.530
		24	0.690	1.380
	280	16	0.454	1.100
		20	0.563	0.542
		24	0.684	1.270

Table 8: It shows results of ruggedness for method 1 and 2

Method	Observations	Analyst 1	Analyst 2
Method 1	Absorbance	0.542	0.538
	Mean, n= 6 (average of 6 determinations)	0.546	0.538
	SD	0.0035	0.002
	% RSD	0.643	0.371
Method 2	Absorbance	0.560	0.560
	Mean, n= 6 (average of 6 determinations)	0.562	0.563
	SD	0.0020	0.0030
	% RSD	0.373	0.539

Table 9: It shows results of assay of marketed formulation of OND (ORNI 500)

Tablet formulation (ORNI 500)	Label claim (mg/tab)	% label claim (average of three determinations±SD)	% RSD	SE
Method 1 (Solvent: Methanol)	500	99.75±0.515	0.517	0.297
Method 2 (Solvent: 0.1 N HCl)	500	96.67±0.362	0.375	0.375

t-calculated value: 0.0203; F-calculated value: 0.159, t-tabulated value (P = 0.05): 1.812; F-tabulated value (P = 0.05, df_1= 2, df_2= 2): 19.00

DISCUSSION

The present study involved the development and validation of a UV-Spectrophotometric method for analysis of OND in methanol and 0.1 N HCl. The λ_{max} of OND was observed to be 311 nm and 277 nm in methanol and 0.1 N HCl respectively. The drug was found to be soluble and stable in both the solvents. The proposed methods obeyed Beer's law in the concentration ranging from 3-18 µg/ml and 10-35 µg/ml respectively. The LOD and LOQ for method 1 were obtained as 0.019 µg/ml and 0.041 µg/ml, respectively, and for method 2; it was obtained as 0.836 µg/ml and 2.53 µg/ml, respectively. The % RSD values of recovery and precision

experiments were within limits for both the methods indicating high accuracy and precision of the methods. The % recovery values were obtained as 101.75 and 96.12 for method 1 and 2 respectively. The methods were also found to be unaffected by minor changes in operating conditions of analysis such as wavelength and concentration. The methods were found to be specific, and no interference from sample matrix was observed as shown by high percent recovery values. The ruggedness of the methods was also established as the % RSD values obtained between two sets of data by different analysts was found to be well within limits. The assay results on marketed formulation obtained by the proposed methods were statistically evaluated and compared by using tests of significance such as Students t-test and F-test. No significant difference was found to exist between the two proposed methods.

CONCLUSION

The present study enabled two simple, rapid, precise and accurate quantitative methods for analysis of OND in bulk and commercial formulations. No tedious extraction procedures or extensive treatment of samples is required, which allows for greater ease of application of the method. The statistical comparison of the methods showed that the two methods are not significantly different. Hence, the methods can be applied to quality control of OND in bulk as well as in its dosage forms.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. http://www.genericpedia.com/generic/ornidazole/. [Last accessed on 10 Nov 2015].
2. http://www.drugs.com/international/ornidazole.html. [Last accessed on 10 Nov 2015].
3. https://en.wikipedia.org/wiki/Ornidazole. [Last accessed on 10 Nov 2015].
4. Boeckh M, Lode H, Deppermann KM, Greisen S, Shokry F, Held R. Pharmacokinetics and serum bactericidal activities of Quinolones in combination with clindamycin, metronidazole, and ornidazole. Antimicrob Agents Chemother 1990;34:2407-14.
5. http://www.medsafe.govt.nz/consumers/cmi/a/arrowornidazole.pdf.html. [Last accessed on 10 Nov 2015].
6. http://www.healthplus24.com/drugs/ornidazole.aspx.html. [Last accessed on 10 Nov 2015].
7. Mazumder R, Nath LK, Giri TK, Choudhuri PK, Kar AK, Sarkar MK. Spectrophotometric method development and determination of Ornidazole in bulk and tablet dosage form. Int J PharmTech Res 2011;3:153-6.
8. Mubeen G, Prakash V, Somashekar PL, Kadri U. Spectrophotometric method for determination of Ornidazole. Int J Pharm Chem Res 2009;1:318-21.
9. Gandhi VM, Nair SB, Menezes SB, Narayan R. Development of a UV-Spectrophotometric method for the quantitative estimation of Ofloxacin and Ornidazole in combined liquid oral dosage form by simultaneous equation method. Int J Res Pharm Chem 2013;3:6-11.
10. Kaur S, Kaur L. Spectrophotometric method for simultaneous estimation of Ornidazole and Cucurmin in pure form. J Pharm Innovation 2014;3:1-4.
11. Krishna JR, Sandhya BN, Huidrom S, Prasad VVLN. Development and validation of UV spectrophotometric method for the simultaneous estimation of ciprofloxacin hydrochloride and ornidazole in combined pharmaceutical dosage form. J Adv Pharm Edu Res 2014;4:405-8.
12. Natraj KS, Suvarna Y, Prasanti G, Saikumar SV. UV Spectrophotometric method development and validation for simultaneous estimation of ciprofloxacin and ornidazole in tablet dosage form. Int Res J Pharm 2013;4:178-81.
13. Dhandapani B, Thirumoorthy N, Rasheed SH, Kotaiah MR, Anjaneyalu N. Method development and validation for the simultaneous estimation of Ofloxacin and Ornidazole in tablet dosage form by RP-HPLC. Int J Pharm Sci Res 2010;1:78-83.
14. Maheshwari RK, Srivastav VK, Prajapat RP, Jain A, Kamaria P, Sahu S. New spectrophotometric estimation of ornidazole tablets employing urea as a hydrotropic solubilizing additive. Int J Pharm Sci 2010;72:258-61.
15. Akhtar J, Shrivastava B, Bhatt P, Patel A, Thakur V. Simultaneous estimation of Ofloxacin and Ornidazole in the synthetic mixture by Q-Analysis UV-spectrophotometric method. Asian J Pharm Life Sci 2011;1:71-5.
16. Patel SA, Patel NM, Patel MM. Simultaneous spectrophotometric estimation of ciprofloxacin and ornidazole in tablets. Int J Pharm Sci 2006;68:665-7.
17. Nalini CN, Ramachandran S, Kavitha K, Harikrishna. Simultaneous determination of Ofloxacin and Ornidazole in tablets by spectrophotometry and reverse phase HPLC. Res J Pharm Biol Chem Sci 2011;2:693-708.
18. Puranik M, Bhaswar DV, Rathi P, Yeole PG. Simultaneous determination of Ofloxacin and Ornidazole in solid dosage form by RP-HPLC and HPTLC techniques. Int J Pharm Sci 2010;72:513-7.
19. Chepurwar SB, Shirkhedkar AA, Bari SB, Fursule RA, Surana SJ. Validated HPTLC method for simultaneous estimation of levofloxacin hemihydrate and ornidazole in pharmaceutical dosage form. J Chromatogr Sci 2007;45:531-6.
20. ICH guidelines, Validation of analytical procedures: text and methodology, Q2A (R1) Nov; 2005.