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Development and Validation of RP-HPLC Method for Simultaneous Determination of Ofloxacin and Ornidazole In Infusion

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ABSTRACT

A simple reverse phase liquid chromatographic method has been developed and subsequently validated for simultaneous determination of Ofloxacin and Ornidazole in infusion dosage form. The separation was carried out using a mobile phase containing methanol and buffer (equal proportion of 0.01M orthophosphoric acid and 0.01M sodium phosphate monobasic dihydrate) with pH 4.00 adjusted by 20% of triethylamine in the ratio of 60:40 v/v. The column used was HiQ Sil C_{18} (150 mm x 4.6 mm i.d, 5 µ) with flow rate of 1 mL / min using UV detection at 300 nm. The described method was linear over a concentration range of 1.25-10 µg/mL (r^2>0.9991) for Ofloxacin and 3.12-25 µg/mL (r^2>0.9992) for Ornidazole. Separation was achieved within 5 min. The mean % recovery was found to be 99.94% for Ofloxacin and 100.27 % for Ornidazole. The limit of detection (LOD) for Ofloxacin and Ornidazole were found to be 0.146 and 0.25 µg/mL respectively. Whereas the limit of quantification (LOQ) for Ofloxacin and Ornidazole was 0.44 and 0.77 µg/mL respectively. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise, accurate and cost effective which is useful for the routine determination of Ofloxacin and Ornidazole in bulk drug and in its infusion.

Keywords: Infusion, Ofloxacin, Ornidazole, RP-HPLC

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INTRODUCTION

Ofloxacin is a fluoroquinolone derivative. chemically, it is (±)-9-fluoro-2, 3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido-[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid[1]. It is official in IP, BP[2], USP[3] and EP[4]. Ofloxacin is characterized by a good pharmacokinetic profile. It is used in the treatment of urinary tract, prostate, skin, and respiratory tract infections. It is also used to treat certain sexually transmitted diseases. Ofloxacin is more active than Ciprofloxacin against *Chlamydia trachomatis*. It is also active against *M. Laprae* [5]. Literature survey reveals that several methods were used for determination of Ofloxacin including spectrophotometric method, atomic absorption spectroscopy[6-9], spectroflurometry[10] and HPLC[11-14].

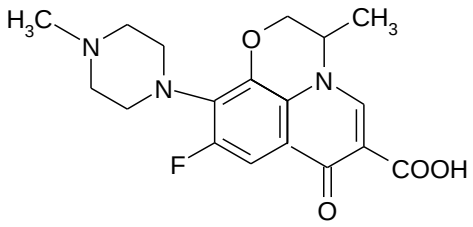

Figure1: Chemical structure of Ofloxacin

Ornidazole is chemically 1-Chloro-3-(2-methyl-5-nitro-1-4-imidzole-1-yl)propan-2-ol[15] and is used in the treatment of susceptible protozoal infections and also in anaerobic bacterial infections. It has been used for amebic liver abscesses, duodenal ulcers, giardiasis, intestinal lambliasis and vaginitis[16-17]. Ornidazole has recently been used with success in patients with active Crohn's disease[18]. Literature survey reveals that several methods were used for determination of Ornidazole by UV[8,9,19], Voltametry[20] and HPLC[21-24] alone and in combination with other drugs. However, there is no method available for their simultaneous estimation of Ofloxacin and Ornidazole in infusion dosage form.

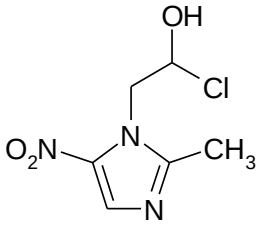

Figure 2: Chemical structure of Ornidazole

Fixed dose combination of Ofloxacin 200 mg and Ornidazole 500 mg is available in tablets as well as infusion dosage form in the market. The present work describes a simple, precise and accurate reversed phase HPLC method for the simultaneous estimation of Ofloxacin and Ornidazole in Infusion dosage form. The method was validated according to procedures and acceptance criteria based on ICH guidelines[25].

MATERIALS AND METHODS

Reagents and Materials

Methanol (HPLC grade) was procured from Thomas baker Chemicals, Mumbai. Sodium phosphate monobasic dihydrate (AR grade) and orthophosphoric acid (AR grade) were purchased from SISCO Research Lab. Mumbai and Poona Chemicals, Pune respectively. Ofloxacin and Ornidazole was obtained as a gift sample from Alkem laboratories Ltd, Mumbai and Macleods Pharmaceuticals Ltd, Mumbai respectively.

Instrumentation

JASCO HPLC 2000 series was used for analysis. The method was carried out on HiQ Sil C_{18} (150 mm × 4.6 mm i.d, 5 µm) column as stationary phase. Borwin software provided by JASCO 2000 series was used throughout this experiment. The injection volume was 20 µL.

Preparation of mobile phase

The mobile phase comprised of methanol and buffer (equal proportion of 0.01M orthophosphoric acid and 0.01M sodium phosphate monobasic dihydrate) with pH 4.00 adjusted by 20% of triethylamine in the ratio of 60:40 v/v. The mobile phase was filtered through 0.45 µm membrane filter and was degassed before use.

Preparation of Ofloxacin and Ornidazole standard stock solution

A stock solution of Ofloxacin and Ornidazole (1000 µg/mL) was prepared by taking 10 mg of each drug, accurately weighed in separate 10 mL volumetric flask. They were dissolved in 2.5 mL of mobile phase and then the volume was made by mobile phase up to the mark. For each drug, appropriate aliquots were pipette out from the standard stock solution into a series of 10 mL volumetric flasks to get a concentration of 1.25, 2.5, 5, 7.5, 10 µg/mL of Ofloxacin 3.12, 6.25, 12.5, 18.75, 25 µg/mL of Ornidazole.

Preparation of sample solution

A stock solution of sample (1000 µg/mL) was prepared by directly taking 5 mL of dosage form in separate 10 mL volumetric flask and then volume was made up to the mark with mobile phase. From this stock solution prepared a middle concentration (i.e. 5 µg/mL of Ofloxacin and 12.5 µg/mL of Ornidazole) and 20 µL injected and peak area of the sample solution was obtained from the software.

Determination of analytical wavelength

The standard solution of Oflocaxin and Ornidazole were injected under the chromatographic condition described above. The elution showed reasonable good response at 300 nm using UV

detector. So both drugs were detected at the common analytical wavelength.

Validation of Proposed HPLC method

System suitability

System suitability tests were performed including resolution, tailing factor, number of theoretical plates, capacity factor etc.

Linearity

The developed method has been validated as per ICH guidelines. Every 20 µL of the solution of Ofloxacin in concentration range of 1.25 to 10 µg/mL and that for Ornidazole in concentration range of 3.12 to 25 µg/mL was injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curves of Ofloxacin and Ornidazole were obtained by plotting the peak area ratio versus the applied concentrations of Ofloxacin and Ornidazole.

Precision

Repeatability of the method was checked by injecting triplicate injections of the solution 5.0 µg/mL and 12.5 µg/mL of Ofloxacin and Ornidazole respectively. Variability of the method was studied by analyzing the solution on the same day (intra-day precision) and on three different days (inter-day precision).

Recovery studies

Recovery experiments were carried out to check for the presence of positive or negative interferences from excipients present in the formulation and to study the accuracy and precision of the method. Recovery experiment was performed by the standard addition method. The recovery of the added standard was studied at three different levels viz 80%, 100% and 120% of the estimated amount of the drug. Each set of recovery of added standard was calculated.

The Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated as 3.3 ∂/S and 10 ∂/S respectively as per ICH guidelines, where ∂ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

Robustness

To determine the robustness of the method, experimental conditions such as the composition of the mobile phase, pH of the mobile phase and flow rate of the mobile phase were altered and the chromatographic characteristics were evaluated by calculating % RSD.

Analysis of Ofloxacin and Ornidazole in Dosage form

The response of sample solution was measured at 300 nm under the chromatographic condition mentioned above for quantitation of Ofloxacin and Ornidazole. The amount of the Ofloxacin and

Ornidazole present in the sample solution were determined by fitting the responses into the regression equation for Ofloxacin and Ornidazole.

RESULTS AND DISCUSSION

Method Development and optimization

The method developed was carried out on HiQ Sil C_{18} (150 mm × 4.6 mm i.d, 5 µm) column as stationary phase and various mobile phases were prepared by mixing solvents like methanol, acetonitrile and buffer (60:40) v/v ratio. The buffer consists of equal volume of 0.01M ortho phosphoric acid and 0.01M sodium phosphate monobasic dihydrate, pH adjusted to (4.0 ±0.2) with triethylamine. The prepared mobile phase was filtered through a Millipore 0.45 µm membrane filter and ultrasonically degassed prior to use. The detection wavelength was set at 300 nm. The elution was done at a flow rate of 1.0 mL/min under ambient condition.

Under these chromatographic conditions, Ofloxacin and Ornidazole peaks were well resolved and their retention time were found to be 2.2 and 3.8 respectively. A typical chromatogram of the drugs is illustrated in Figure 3. The % assay or average amount of Ofloxacin and Ornidazole found to be 101.60% and 100.89% respectively (Table 1). System suitability tests including resolution, tailing factor, number of theoretical plates, capacity factor were carried out and results are shown in Table 2. All of these results were acceptable in their limits defined by official guidelines[26].

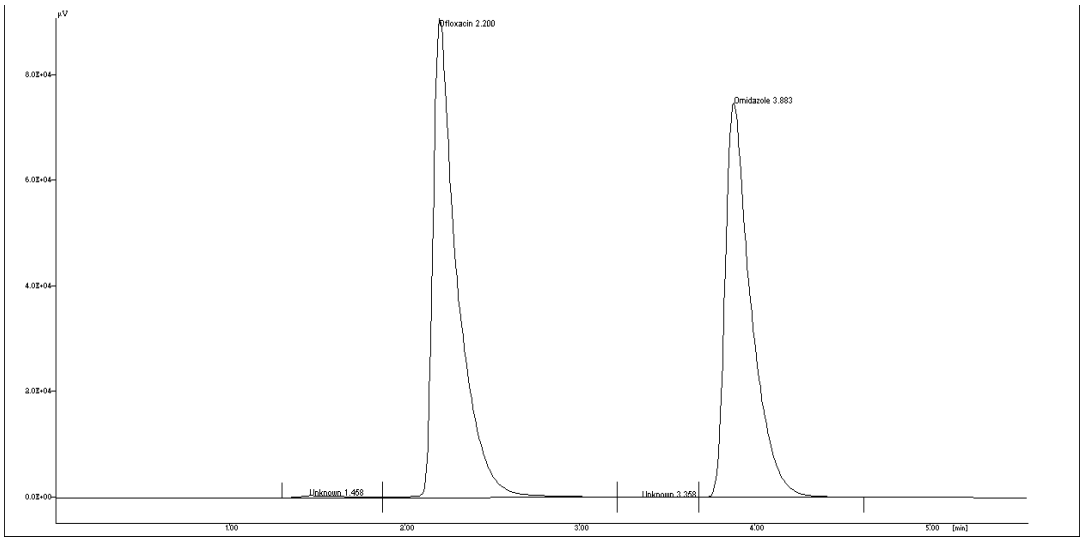

Figure 3: Representative HPLC chromatogram of Ofloxacin and Ornidazole.

Table 1. Assay of Ofloxacin and Ornidazole

Drug	Labelled claim (mg)	Amount found(mg)	%RSD	% Assay
Ofloxacin	5.0	5.08	0.9	101.60
Ornidazole	12.50	12.61	1.5	100.89

Table 2. System suitability test for Ofloxacin and Ornidazole

Parameter	Ofloxacin	Ornidazole
Resolution (R)	2.57	4.79
Asymmetry (A_s)	1.87	1.81
No. of theoretical plates (N)	2127	3457
Capacity factor (K')	2.2	3.8

Validation of Proposed method

Linearity

Calibration graph was found to be linear at range 1.25 to 10 µg/mL, 3.13 to 25 µg/mL for the Ofloxacin and Ornidazole respectively. Regression analysis of the calibration data for Ofloxacin and Ornidazol showed that the dependent variable (peak area) and the independent variable (concentration) were represented by the equations. For Ofloxacin the correlation coefficient (r^2) obtained was found to be 0.9991 and 0.9992 for Ornidazole(Table 3).

Table 3: Regression Analysis Data and Summary of Validation Parameters

Parameters	Ofloxacin	Ornidazole
Detection wavelength(nm)	300 nm	
Linearity range (µg/mL)	1.25 -10	3.12-25
Slope	10815	41254
Intercept	17404	1863
r^2	0.9991	0.9992
LOD (µg /mL)	0.146	0.25
LOQ (µg /mL)	0.44	0.77
Intra day (% RSD)*	0.8	0.7
Inter day (% RSD)*	1.2	0.7
Repeatability (% RSD)*	0.8	0.6
Mean Recovery	99.94	100.27%

Precision

The repeatability results were found to be 0.8 % and 0.6 % for Ofloxacin and Ornidazole respectively. The results obtained inter-day precisions (%RSD) were 1.2% and 0.7% respectively for both Ofloxacin and Ornidazole. The results obtained for intra-day precision (% RSD) were 0.8 % and 0.7% respectively both Ofloxacin and Ornidazole.

Recovery

The mean % recovery was found to be 99.94% for Ofloxacin and 100.27 % for Ornidazole. The amounts recovered and the values of percent recovery were calculated for Ofloxacin and Ornidazole separately, results are shown in Table 4 and Table 5.

LOD and LOQ

The limit of detection (LOD) for Ofloxacin and Ornidazole were found to be 0.146 and 0.25 µg/mL respectively. Whereas the limit of quantification (LOQ) for Ofloxacin and Ornidazole

0.44 and 0.77 µg/mL respectively.

Table 4: Recovery studies for Ofloxacin

Conc. of Std. Solution (µg/mL)	Conc. of sample solution (µg/mL)	Amount found (µg/mL)	% Recovery	% RSD
2.5	2	4.48	99.61	0.8
2.5	2.5	5.006	100.12	0.9
2.5	3	5.5	100.09	1.2

Table 5: Recovery studies for Ornidazole

Conc. of Std. Solution (µg/mL)	Conc. of sample solution (µg/mL)	Amount found (µg/mL)	% Recovery	% RSD
6.25	5	11.17	99.31	1.3
6.25	6.25	12.66	101.32	1.1
6.25	7.5	13.77	100.18	0.8

Robustness

The standard deviation of peak areas was calculated for each parameter and % RSD was found to be less than 2 %.

CONCLUSION

As the number of multi-component formulations are increasing in various dosage form, hence The present work was undertaken with an aim to meet these challenges of analytical chemist. RPHPLC method was developed for Ofloxacin and Ornidazole in infusion dosage form and was validated as per ICH guidelines. The results of the study shows that the developed RP-HPLC method is simple, rapid, precise, accurate and cost effective, which is useful for the routine determination of said drug in bulk as well as its infusion dosage form.

REFERENCES

1. Indian Pharmacopoeia, Vol. III , New Delhi, The controller Publication, Govt. of India; 2007:1468-1470.
2. British pharmacopeia, Vol. II, London, The British Pharmacopoeia Commission;2004**:**1412-1413.
3. United State Pharmacopoeia, Vol. II, Rockville, United States Pharmacopoeial convention;2005: 1413-1415.
4. European Pharmacopoeia, Vol. II, Strasbourge, Directorate for the quality of Medicines of Council of Europe; 2005: 2131-2132.
5. Monk JP and Campoli-Richards DM. Ofloxacin: A review of its antibacterial activity, pharmacokinetic properties and therapeutic uses. Drugs. 1987; 33: 346-391.
6. Shivkumar CV, Gunturu C, Indrakanti M. Simultaneous Estimation of Ofloxacin and

Ornidazole by UV spectroscopy. Inter. J. Pharmacy and Tech 2011; 3; 3083-3091.

7. Attimarad M. Rapid RP HPLC Method for quantitative determination of Lornoxicam in tablets. J Basic and Clinical Pharm 2010; 5: 51- 57.
8. Rege PV, Sathe PA, Salvi VS, Shotri CK. Simultaneous Spectrophotometric Estimation of Ofloxacin and Ornidazole from Combined Tablet Dosage form. Int. J. Pharma World Research. 2011;2: 1-24.
9. Bhusari KP, Chaple DR. Simultaneous Spectrophotometric Estimation of Ofloxacin and Ornidazole in Tablet Dosage Form. Asian J. Res Chem 2009; 2: 60-62.
10. Hesham S. Spectrofluorimetric, Atomic Absorption Spectrometric and Spectrophotometric Determination of Some Fluoroquinolones. American Journal of Applied science 2005; 2: 719-729.
11. Shinde SR, Bhoir IS, Pawar NS, Yadav BS, Bhagwat MA. Simultaneous Estimation of Satranidazole and Ofloxacin in Tablet Dosage form by High Performance Liquid Chromatography. E. J. Chem 2010; 7: 198-202.
12. Khandagle KS, Gandhi SV, Deshpande PB, Gaikwad NV. Simple and Sensitive RP-HPLC method for Simultaneous estimation of Cefixime & Ofloxacine in combined dosage form. Int. J. Pharmacy and Pharmaceutical Sciences 2011; 3: 46-48.
13. Kotaiah MR, Shaikh HR, Rao YN, Venkateswarlu Y, Konda RK. Simultaneous estimation of ofloxacin and tinidazole in tablet dosage form by RP-HPLC. Res. J. Pharm. Bio. Chemical Sciences 2011; 2: 53-57.
14. Narayana LK, Manohara YN, Appala RS. Development and validation of RP-HPLC method for the estimation of Nitazoxanide & Ofloxacin in combined tablet dosage form. Indian Drugs 2006; 43: 503-506.
15. Maryadele JO Neil. The Merck Index: An Encyclopedia of chemicals, drugs and biologicals. 14th ed., whitehouse station, New Jersey: published by Merck Research Laboratories, Division of Merck and Co., Inc; 2006:1184.
16. Lamp KC, Freeman CD, Klutman NE, Lacy MK. Pharmacokinetics and Pharmacodynamics of Nitroimidazole antimicrobials. Clin. Pharmacokinet 1999; 36: 353-373.
17. Schwartz DE, Jeunet F. Pharmacokinetics of ornidazole in patients with renal insufficiency; influence of haemodialysis and peritoneal dialysis Chemotherapy 1976; 22: 19-29.
18. Triantafillidis JK, Nicolakis D, Antoniou A, Hereti I. Bioavailability of Ornidazole. Am.

J. Gastroenterol 2001; 96: 254-255.

19. Kastur VS, Bhagat AD, Puro NC, More PS, Bhandari NK. Spectrophotometric method for simultaneous estimation of ofloxacin and Cefixime in tablet dosage form. Indian Drugs 2004; 41: 51-53.
20. Dhandapani B, Thirumoorthy N, Shaik RH, Rama M, Anjaneyalu N. Method Development and Validation for the Simultaneous Estimation of Ofloxacin and Ornidazole in Tablet Dosage Form By RP-HPLC. Inter J Pharma Sciences and Res 2010; 1: 78-83.
21. Kabra PV, Kimbahune RV, Puranik MP. Simultaneous Estimation of Gatiloxacin, Ornidazole and its Isomer by Reverse Phase High Performance Liquid Chromatography. Int. J. ChemTech. Research 2010; 2: 928-931.
22. Wankhede SB, Prakash A, Chitlange S S. Simultaneous estimation for Norfloxacine & Ornidazole in combined Tablet dosage form. Int. J. ChemTech. Research 2009; 1: 937-940.
23. Ghosh SJ, Darbar S, Chowdhury PP, Chattopadhyay SP, Chakraborty MR. Simultaneous Determination and Validation of Ofloxacin and Ornidazole in Combined Dosage Pharmaceutical Formulation. Int. J. Pharm.Research 2010; 2: 367-374.
24. Singh R, Maithani M, Saraf SK, Gupta RC. Simultaneous estimation of Ciprofloxacin hydrochloride, Ofloxacin, Tinidazole, Ornidazole in tablet dosage form by RP-HPLC method. Eur. J. Anal. Chem 2009; 4: 161-167.
25. International Conference on Harmonization, Q2 (R1), Validation of Analytical Procedure: Text and Methodology: 2005.
26. Validation of Chromatographic Methods: Reviewer Guidance, Center of Drug Evaluation and Research. Rock-ville: U.S. Food and Drug Administration: 1994.