Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2012; 4(4): 52-55

ISSN 0975 1556

Research Article

# Validated Simultaneous Estimation and Development of Levofloxacin and Ornidazole by RP-HPLC Method

*Shafrose Syed, Haritha Pavani

*Nimra College of Pharmacy, Nimra Nagar, Ibrahimpatnam, Vijayawada-521456*

**ABSTRACT**

A simple, economic, selective, precise, and accurate Reverse phase High Performance Liquid Chromatography method for analysis of Levofloxacin and Ornidazole, was developed and validated according to ICH guidelines. The quantification of drug was carried out using Hypersil BDS $C_{18}$ 150mm × 4.6mm × 5µm column or its equivalent in isocratic mode, with mobile phase compressing of Buffer: Acetonitrile (75:25) the flow rate was 1.0ml/min and the detection was carried at 315 nm. The retention time and percentage assay for Levofloxacin and Ornidazole was found to be 3.0 and 4.8min, 103.4% and 104.16% respectively. Proposed method was validated for precision, accuracy, linearity range, specificity and robustness.

**Key words:** Levosulpiride and Rabeprazole sodium, RP-HPLC, Validation.

## INTRODUCTION

Levofloxacin is chemically (S)-9-fluoro-2, 3-dihydro-3-methyl-10-(4-methylpiperazin-1-yl -7-oxo-7H-pyrido [1, 2, 3-de]-1, 4-benzoxazine-6-carboxylic acid and official in pharmacopoeia. It is used to treat the Pneumonia and exacerbations of chronic bronchitis, sinusitis, enteric fevers, Pyelonephritis and Skin/Soft tissue infections.

Ornidazole is chemically 1-chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl) propan-2-ol and official in pharmacopoeia. Ornidazole is an imidazole derivative which is used to treat some protozoan infections.

Literature review reveals that UV[(1)], HPLC[(2,3,4)] methods for Levofloxacin alone or in combined dosage forms and various UV[(5)], HPTLC[(6)], HPLC[(7,8,9)] methods for Ornidazole alone or in combined dosage forms. The aim of the present study was to develop accurate, precise and selective reverse phase HPLC methods for the simultaneous analysis of Levofloxacin and Ornidazole.

## MATERIAL AND METHODS

Chemicals and Reagents: Levofloxacin and Ornidazole were obtained as gift samples from Spectrum Labs, Hyderabad, Andhra Pradesh. We used HPLC grade acetonitrile, water and AR grade triethylamine and ortho phosphoric acid.

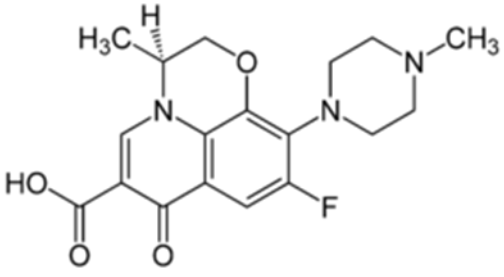


**Fig.1: Levofloxacin**

Instrumentation: A HPLC (Waters 2996) with UV/VIS Detector/PDA detector and Hypersil BDS $C_{18}$ 150mm × 4.6mm × 5µm column was used. The HPLC system was equipped with Empower2 software for data processing.

Chromatographic Condition: The mobile phase containing Buffer: Acetonitrile (75:25) was found to resolve Levofloxacin and Ornidazole. Ortho phosphoric acid was used for pH adjustment of buffer to 3.15. The mobile phase was filtered through 0.45 nylon filter and then ultrasonicated for 30 min. The flow rate was set to1.0ml/min. The drug shows good absorbance at 315nm, which was selected as wavelength for further analysis.

Buffer Preparation: Accurately transfer 1.0ml of triethylamine into 500ml of distilled water and adjust $P^H$ with ortho phosphoric acid to 3.15. Filter the solution through 0.45µm nylon filter.

Preparation of Mobile Phase: Preparedly filtered and degassed mixture of buffer and Acetonitrile in the ratio of 75:25 v/v

Diluent solution: HPLC grade water was used as diluent.

Preparation of Standard solution: Accurately weighed and transferred about 25 mg of Levofloxacin and 50mg of Ornidazole working standard into a 50ml volumetric flask add 30 ml of diluent, sonicated for 15 minutes and make

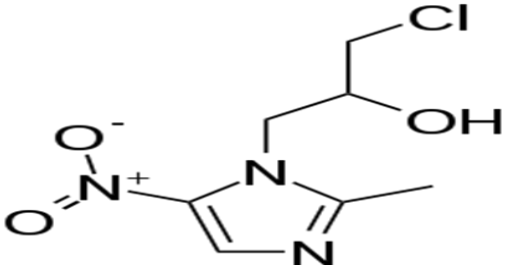


**Fig.2: Ornidazole**

*Author for correspondence: E Mail :sdshafrose.syed@gmail.com*

Table.1: System suitability data

| Parameter | Levofloxacin | Ornidazole |
|---|---|---|
| Tailing Factor | 1.43 | 1.06 |
| Theoretical Plates | 4118 | 9074 |
| %RSD of Peak area | 1.49 | 1.58 |

Table.2: System Precision Data

| Parameter | Levofloxacin | Ornidazole |
|---|---|---|
| Mean peak area | 2933568 | 7290367 |
| SD | 45062.81 | 120922.5 |
| %RSD of Peak area | 1.54 | 1.66 |

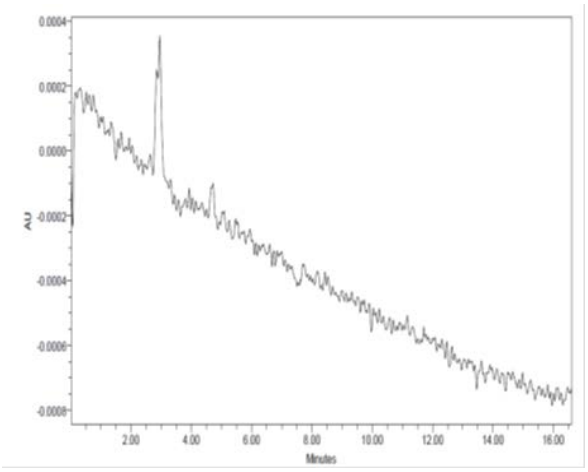


Fig.3: Chromatogram of Blank

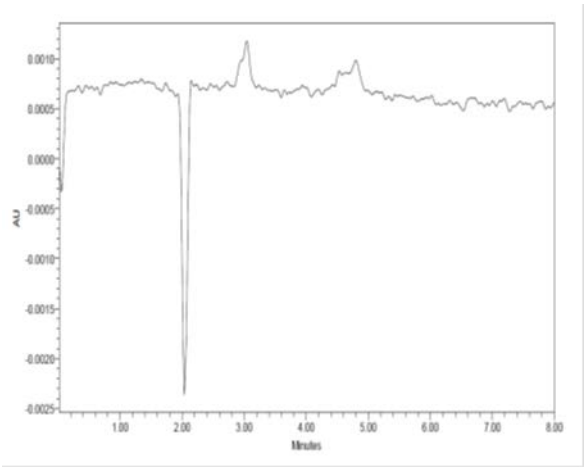


Fig.4: Chromatogram of Placebo

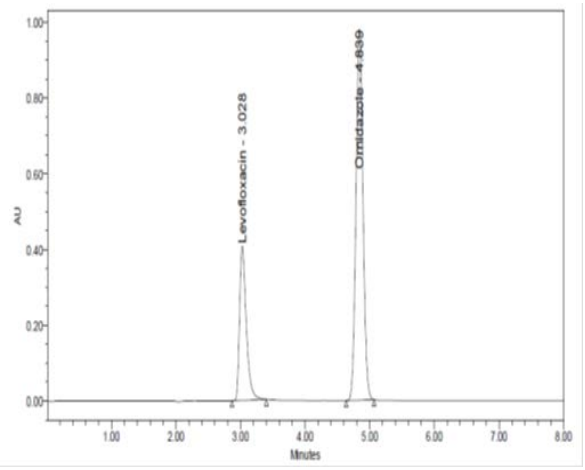


Fig.5: Chromatogram of Standard

up to the mark with diluent (this is standard stock solution).

Transferred 5.0 ml of the above solution in to 10ml volumetric flask made up to the mark with diluent. Filter the solution through 0.45µm nylon filter.

Preparation of Sample solution: Crush 20 tablets and transferred accurately weighed powder equivalent to 25mg of Levofloxacin and 50mg of Ornidazole into 50ml volumetric flask add 30ml of diluent sonicate to dissolve and make up to the volume with diluent. Filter the solution through 0.45µm nylon filter. Transfer 5ml of above solution into 10ml volumetric flask and make up to the volume with diluent.

**METHOD VALIDATION**

1) System Suitability: System Suitability was performed by injecting six replicate injections of standard solutions of Levofloxacin and Ornidazole at 100% and measured the retention time, theoretical plates and tailing factor.

2) System Precision[10]: To assess the system precise for conducting validation inject six replicates of standard preparations of 100% level for Levofloxacin & Ornidazole and expressed as %RSD of peak area.

3) Specificity: To demonstrate that diluents and placebo are not interfering with analytic peak. Solutions of Standard and Sample were prepared as per test procedure and injected into the HPLC system.

Table.3: Repeatability data

| Parameter | Levofloxacin | Ornidazole |
|---|---|---|
| Mean | 103.4 | 104.16 |
| SD | 1.673 | 1.75 |
| %RSD | 1.62 | 1.68 |

Table.4: Linearity Data

| Level | Concentration (µg/ml) | | Peak Area | |
|---|---|---|---|---|
| | Levofloxacin | Ornidazole | Levofloxacin | Ornidazole |
| 50% | 125 | 250 | 2041934 | 5049810 |
| 80% | 200 | 400 | 3245157 | 8009532 |
| 100% | 250 | 500 | 4109179 | 10296344 |
| 120% | 300 | 600 | 5100572 | 12658500 |
| 150% | 375 | 750 | 6201130 | 15352210 |
| Slope | - | - | 16901 | 20969 |
| Intercept | - | - | -85717.7 | -211192.5 |
| Correlation Coefficient | - | - | 0.999 | 0.999 |

Table.5: Accuracy data

| Drug | %Level | Mean Peak Area | % Mean recovery | %RSD |
|---|---|---|---|---|
| Levofloxacin | 50% | 2088820 | 98.36 | 0.14 |
| | 100% | 4134444 | 97.34 | 0.27 |
| | 150% | 6273303 | 98.46 | 0.25 |
| Ornidazole | 50% | 5227760 | 98.55 | 0.21 |
| | 100% | 10329081 | 97.36 | 0.25 |
| | 150% | 15822107 | 99.42 | 0.29 |

Table.6: Robustness data

| Parameter | Retention Time | | Peak Area | |
|---|---|---|---|---|
| | Levo | Orni | Levo | Orni |
| Actual | 2.988 | 4.807 | 4566863 | 10934227 |
| High Flow Rate | 2.383 | 3.805 | 3454489 | 8533535 |
| Low Flow Rate | 3.865 | 6.214 | 5123168 | 12920894 |
| Low Buffer | 2.319 | 3.528 | 4252046 | 10524590 |
| High Buffer | 4.059 | 6.187 | 4224635 | 10139716 |
| High Temperature | 2.796 | 4.479 | 4206938 | 10413837 |

4) Method Precision[10]: Method Precision was measured in terms of repeatability of application and measurement. Repeatability of sample application was carried out using six replicates of the same sample concentration.

5) Linearity[11]: The linearity of the HPLC method was demonstrated for Levofloxacin and Ornidazole solutions ranging from 50% to 150% of standard concentrations.

6) Accuracy (%Recovery) [12]: %Recovery studies were carried out at three different levels of 50%, 100% and 150% of standard solution (i.e. Levofloxacin and Ornidazole API spiked to the placebo) in triplicate in each level.

7) Robustness[10, 13]: The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate, buffer composition and column temperature which may differ but the responses were still within the specified limits.

**RESULTS AND DISCUSSION**

Optimization of the mobile phase was performed based on resolution, asymmetric factor and peak area obtained for Levofloxacin and Ornidazole. The Mobile phase Acetonitrile: buffer (phosphate buffer, 75:25) was found to be satisfactory and gave symmetric and well resolved peak for Levofloxacin and Ornidazole. Results were summarized in Table.no.1.

The percentage relative standard deviation for peak area of Levofloxacin and Ornidazole in system precision was found to be 1.54 and 1.66 which indicate that the test method meets the acceptance criteria. Results were summarized in Table.no.2.

Good resolution obtained between the analytes Levofloxacin and Ornidazole peaks and no interference of blank and placebo observed at the retention times of Levofloxacin and Ornidazole & chromatograms were shown in Fig.3, 4 & 5.

Precision was determined & the results are represented in the form of %RSD for assay of Levofloxacin and Ornidazole were found to be below 2% & shows that the test method was highly precise and results given in

Table.3. The correlation coefficient ($r^2$) for Levofloxacin and Ornidazole was found to be 0.999 & 0.999 and shows good linearity. The data of the calibration curve was given in Table.4.

The % mean recovery for Levofloxacin and Ornidazole were found to be in the range of about 97.34%-98.46% and 97.36%-99.42%.The results were summarized in Table.5.

As part of the robustness, deliberate changes in the flow, column temp & buffer composition was made to impact on the method. RT and Peak area were significantly changed but within the acceptance limit and results given in Table.6.

**CONCLUSION**

It can be concluded that the proposed RP-HPLC method is accurate, precise, sensitive, specific, robust and reproducible for the simultaneous analysis of Levofloxacin and Ornidazole with less tailing and is also economical.

**REFERENCES**


1. T. Manish Kumar*, Gurrala Srikanth, J. Venkateshwar Rao, KRS. Sambasiva Rao, Development and validation of HPLC-UV method for the estimation of levofloxacin in human plasma, Int J Pharm Pharm Sci, Vol 3, Issue 2, 2011, 247250.
2. Teja kumar.R*, A. Chitra, R. Vijay Amrithraj, N. Senthil kumar, New RP-HPLC Method development and validation for estimation of levofloxacin in tablet dosage form, Journal of Global Trends in Pharmaceutical Sciences Vol.2, Issue 3, pp -264-276, July -Sept 2011.
3. Rekha rajeevkumar*, Nagavalli D, Rajeev Kumar P and Devi T, RP-HPLC Method Development and Validation for the Simultaneous Estimation of Levofloxacin hemihydrate and ornidazole in Tablets, International Journal of Pharm Tech Research, Vol.1, No.4, pp 1161-1163, Oct-Dec 2009.
4. P. Suresh kumar, S .Navaneetha Krishnan, V. Naveen Kumar, G. Anil Kumar, G. Kiran Kumar, HPLC method development of Levofloxacin by RP-HPLC in its bulk dosage forms , IJRAP 2011.2(6) 1790-1792.
5. Rana Mazumder*, Lila K. Nath, Tapan K. Giri, Prasanta K. Choudhury , Ayan K. Kar, and Manas K. Sarkar, Spectrophotometric method development and determination of ornidazole in bulk and tablet dosage form, International Journal of Pharm Tech Research, Vol. 3, No.1, pp 153-156, Jan-Mar 2011.
6. Satish A. Patel*, Natvarlal J. Patel, Development and validation of HPTLC method for simultaneous estimation of levofloxacin and ornidazole in tablet dosage form, J Pharm Educ Res Vol. 2, Issue No. 2, December 2011, pg.no.78-83.
7. Satish a patel, Development and validation of RP-HPLC method for simultaneous determination of ciprofloxacin and ornidazole in tablets, Int J Curr Pharm Res, Vol 3, Issue 4, 72-75.
8. Sarode M. R.*, Chaple D. R., Nikhade P. A., Yeole M. P. Development and validation of RP-HPLC method for simultaneous estimation of cefixime and ornidazole in tablet dosage form, International Journal of Universal Pharmacy and Life Sciences 2(4): July-August 2012, 258-263.
9. B.Dhandapani*, N.Thirumoorthy, Shaik Harun Rasheed, M.Rama kotaiah and N.Anjaneyalu, Method development and validation for the simultaneous estimation of ofloxacin and ornidazole in tablet dosage form by RPHPLC, International Journal on Pharmaceutical and Biomedical Research Vol. 1(1), 2010, 42-48.
10. Snyder LR, Kirkland JJ, Glajch JI. Practical HPLC Method Development. 2nd ed.; 1997.
11. ICH QIA (R2). Stability Testing of New Drug Substances and Products. 2003
12. ICH, Q2B.Validation of Analytical Procedure: Methodology. International Conference on Harmonization, IFPMA, Geneva, 2005.
13. Validation of Analytical Procedure: Methodology, ICH Harmonized Tripartite Guidelines. 1996. p. 1-8.