RESEARCH ARTICLE

Development and Validation of RP-HPLC method for the Simultaneous estimation of Ciprofloxacin Hydrochloride and Ornidazole in Combined Pharmaceutical Dosage Form

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J. Adv. Pharm. Edu. & Res.

ABSTRACT

A simple, specific, accurate and precise reverse phase high performance liquid chromatographic method has been developed for the simultaneous estimation of Ciprofloxacin hydrochloride and Ornidazole in pure and combined pharmaceutical dosage form. The quantification of the drug was carried out using Reverse phase phenomenex® Luna 5µ C18 (2) 100A (250 × 4.60 mm i.d) column in isocratic mode with mobile phase containing Acetonitrile and Water (p^H adjusted to 2.5 with O-phosphoric acid) in the ratio of (75:25 % v/v) was used. The flow rate was 1ml/min and the effluents were monitored at 289nm. The retention times of Ciprofloxacin hydrochloride and Ornidazole were found to be 6.319mins and 2.944 mins respectively. The developed method was validated in terms of linearity, accuracy, precision, LOD, LOQ as per ICH guidelines. Due to its simplicity, accuracy and high precision the proposed RP- HPLC method can be used for the simultaneous estimation of both the drugs in its combined pharmaceutical dosage form.

Keywords: Ciprofloxacin hydrochloride, Ornidazole, RP-HPLC, Analytical method development, Validation.

INTRODUCTION

Ciprofloxacin is a broad spectrum anti-biotic active against gram+ve and gram-ve bacteria. It functions by inhibiting DNA gyrase,a type 2 topoisomerase and type IV enzyme necessary to separate bacterial DNA, there by inhibiting cell division.. Chemically Ciprofloxacin is 1-Cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylicacid.Figure1a.Ornidazole is used in the treatment of amoebiasis and other protozoal infections. Ornidazole is chemically 1-chloro-3-(2-methyl-5-nitroimidazole-1-yl)-propan-2-ol Figure. 1b. Literature survey reported that very few analytical methods such as UV Spectroscopy, HPLC have been reported for the simultaneous estimation of both the drugs. The aim of the present study is to develop and validate a simple, precise and accurate and reproducible RP-HPLC method for analysis of ciprofloxacin and Ornidazole in a combined pharmaceutical dosage form and hence a simple and accurate method was developed and validated.

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MATERIALS AND METHODS

Instruments

Shimadzu (Japan) high performance liquid chromatograph equipped with (LC–20 AD pump), LC-20A UV/Vis detector, (rheodyne) 7725 i injection with 20 µl loop, phenomenex® Luna 5µ C18 (2) 100A (250 × 4.60 mm i.d) column and LC solutions software.Shimadzu electronic balance model AX 200 and Ultra Sonicator (Fast clean) model 2k811056 were also used during the analysis.Lab India®, pH meter, Value 1 stage, Vacuum pump.

Materials

Analytically pure samples of Ciprofloxaicn and Ornidazole were obtained as gift samples from CIPLA LTD. Tablets of brand "Cifran-oz"having combination of Ciprofloxacin (500 mg) and Ornidazole (500 mg) manufactured by Ranbaxy Laboratories Ltd was purchased from local pharmacy.

Preparation of mobile phase

The mobile phase was prepared by mixing of acetonitrile with water (p^H 2.5) (75:25v/v) and the water p^H was adjusted to 2.5 using O-phosphoric acid. The mobile phase was sonicated for 5 mins and then it was filtered through a 0.45µ membrane filter paper.

Preparation of standard solutions

Stock solutions were prepared by dissolving 10 mg of Ciprofloxacin, 10 mg of Ornidazole in 10 ml of mobile phase separately. Aliquots of the standard stock solutions of Ciprofloxacin and Ornidazole were transferred into 10 ml volumetric flasks and solution was made up to the volume to yield required concentrations of Ciprofloxacin and OrnidazoleFigure.2.

Preparation of sample solutions

Twenty cifran-oz tablets each containing 500mg of Ciprofloxacin and 500mg of Ornidazole were weighed, average weight was calculated and powdered. A quantity equivalent to 10mg of CPF and ORD was weighed and transferred into 10 ml volumetric flask. It is extracted with mobile phase. The volumetric flask was sonicated for 20 minutes to affect the complete dissolution of the drugs and the solution was made up to the volume with mobile phase and filtered. Suitable aliquots of formulation solution were prepared and injected to HPLC to obtain concentration in the linearity rangeFigure.5.The results are shown in Table.5.

Method Validation

Linearity

Linearity was established by least squares linear regression analysis of the calibration curve. The calibration curves were linear over the concentration range of 2-10 µg/ml for Ciprofloxacin and 6-16µg/ml for Ornidazole. Peak areas were plotted versus respective concentrations and linear regression analysis was performed on the resultant curves. The correlation coefficient were found to be 0.999 for Ciprofloxacin and 0.997 for Ornidazole Figure 3-4. The results are given in Table.1.

Precision:

To check the reproducibility of the method,suitable statistical evaluation was carried out. The concentrations of two drugs were measured three times on the same day at intervals of 1hour and on three different days for intra and inter day study respectively. The standard deviation and Relative Standard Deviation were calculated (RSD) were calculated. The results are shown in Table.2.

Accuracy

Recovery studies of the drug were carried out for determining accuracy parameter. It was done by mixing known quantity of standard drugs with the analyzed sample formulation and the contents were reanalyzed by the proposed method. This was carried out at 50, 100 and 150 % levels. The results are shown in Table.3.

LOD and LOQ

LOD and LOQ were determined by injecting progressively lower concentrations of two drugs. The LOD of Ciprofloxacin and Ornidazole were found to be 0.2299 µg/ml and 0.283 µg/ml respectively. The LOQ of Ciprofloxacin and Ornidazole were found to be 0.6969 µg/ml and 0.85973 µg/ml respectively. The results are shown in Table.4.

RESULTS AND DISCUSSION

A wavelength of 289nm was selected for the present study. Different mobile phase systems in different proportions were tried. From this a mixture water (pH adjusted to 2.5 with Orthophosphoric acid): Acetonitrile (25:75v/v) produced symmetric peak with good resolution for both the drugs. Next, the drugs were chromatographed under different flow rates and 1.0ml/min was selected. The retention times Ornidazole and Ciprofloxacin were found to be 2.944 min and 6.319min, respectively.The developed method was validated as per the ICH guidelines.

CONCLUSION

The proposed RP-HPLC method was found to be simple, precise, accurate and specific for the simultaneous estimation of Ciprofloxacin and Ornidazole in combined pharmaceutical dosage forms. Hence, this method can be easily used for routine quality control analysis of Ciprofloxacin and Ornidazole in pure and its combined dosage form

Table 1: Linearity and Correlation coefficient

Parameters	Ciprofloxacin	Ornidazole
Regression equation	Y= 67737x + 42246	Y=25380x + 5873
Linearity (µg/ml)	2-10	2-10
Correlation coefficient	0.999	0.999

Table 2: Precision studies

Drug	Concentration µg/ml	Intraday Precision n=3 %RSD	Interday Precision n=3 %RSD
Ciprofloxacin	6µg/ml	0.479	0.559
Ornidazole	6µg/ml	1.02	1.48

Table 3: Accuracy

Drug	Amount taken	Amount added	Amount recovered	% Recovery	% *RSD
		250	248.12		
Ciprofloxacin	500	500	497.34	99.52	0.532
		750	748.94		
		250	247.16		
Ornidazole	500	500	498.12	99.42	0.467
		750	748.32		

*Mean of six observations

Table 4: LOD and LOQ studies

Validation parameters	Ciprofloxacin	Ornidazole
LOD (µg/ml)	0.2299	0.283
LOQ (µg/ml)	0.6969	0.85973

Table 5: Analysis of Formulation

Drug	Labelled amount (mg/tablet)	Amount found (mg/tablet)	%Label claim	%*RSD
Ciprofloxacin	500 mg	497.8	99.56	0.652
Ornidazole	500 mg	496.54	99.308	0.254

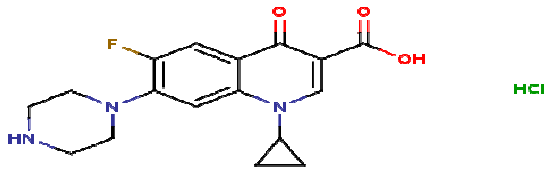

Figure 1a: Structure of Ciprofloxacin hydrochloride

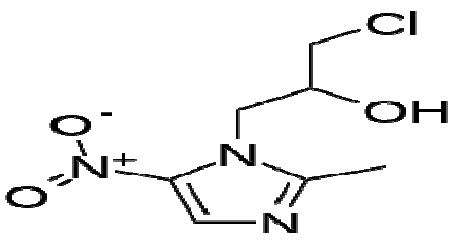

Figure 1b: Structure of ornidazole

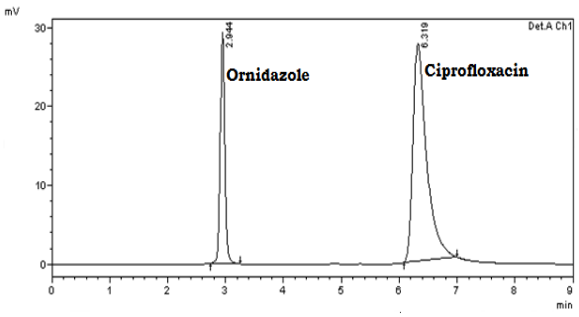

Figure 2: Chromatogram of standard solution (6µg/ml Ciprofloxacin and Ornidazole)

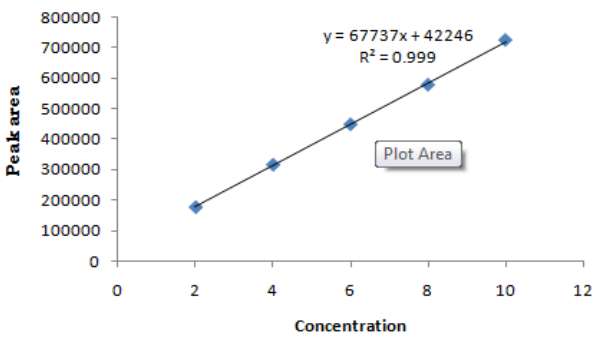

Figure 3: Calibration graph of Ciprofloxacin by RP-HPLC

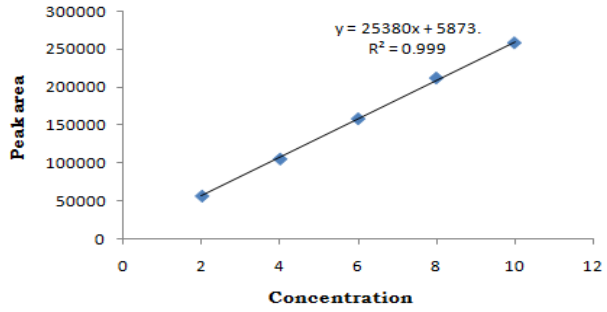

Figure 4: Calibration graph of Ornidazole by RP-HPLC

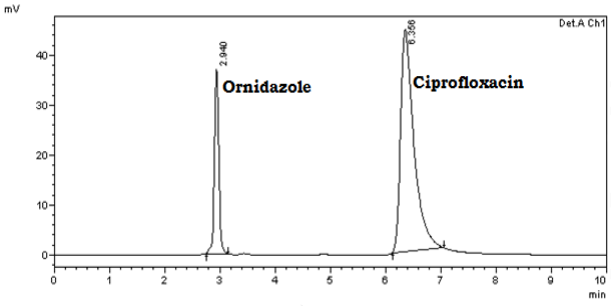

Figure 5: Chromatogram of formulation (6µg/ml Ciprofloxacin and Ornidazole)

Acknowledgement

I am very thankful to School of Pharmacy, Anurag Group of Institutions, Ranga reddy for giving permission to carry out my research work.

REFERENCES

1. Shihn-Sheng Wu, Chih-Yuan Chein,Yen-Hsia Wen. Analysis of Ciprofloxacin by a Simple High-Performance Liquid Chromatography Method. Journal of Chromatographic Science 2008; 46:490-95.
2. Sneha Jansari K, Nirav Patel B, Parag Patel R,Nikita Patel N, Hemant Desai T. Development and validation of stability indicating method for simultaneous estimation of ciprofloxacin hcl and tinidazole using rp-uplc method. ISOR Journal of Pharmacy 2012;2:12-19.
3. Rege P.V, Ramesh Mapari. Simultaneous quantification of Ofloxacin and Ornidazole from combinrd pharmaceutical drug formulation by Hplc. International Journal of Pharma and Bio Sciences 2011;2:51-58.
4. Shafrose Syed, Haritha Pavani. Validated Simultaneous Estimation and Development of Levofloxacin and Ornidazole by RP-HPLC Method. International Journal of Pharmaceutical and Clinical Research 2012; 4: 52-55.
5. Syeda kulsum, Rahul Reddy C,Kanaka Durga M, Padmalatha M. A simple and validated RP-HPLC method for the simultaneous estimation of Tinidazole and Ciprofloxacin in bulk and pharmaceutical dosage forms. International Journal of Research and Development in Pharmacy and Life Sciences 2012-2013;2:238-43.
6. Carolin Nimila,Balan P,Sathiya Sundar R, Ashok Kumar J, Rajasekar S, Simultaneous RP-HPLC Estimation of Ciprofloxacin Hydrochloride and Ornidazole in Tablet Dosage Form. Asian J.Research Chem.2011;4:227-230.
7. Ravi Varma A, Shanmukha Kumar J.V, Mutth Reddy S. Development and Validation of liquid chromatographic method for the Simultaneous Estimation of Ciprofloxacin and Tinidazole in Combined dosage form. International Research Journal of Pharmaceutical and Applied Sciences 2013;3:161-167.
8. Gurudeep R.Chatwal, Sham K.Anand. Instrumental Methods of Chemical Analysis.5th edition.Himalaya Publishing House;2002.
9. Beckett,A.H.,Stenlake,J.B. Practical Pharmaceutical Chemistry 2.4th edition.CBS Publishers and Distributors;1997.

How to cite this article: J. Ramya Krishna*[1], B. Naga Sandhya[1], Sanayaima Huidrom[1], V.V.L.N Prasad[1]; Development and Validation of RP-HPLC method for the Simultaneous estimation of Ciprofloxacin Hydrochloride and Ornidazole in Combined Pharmaceutical Dosage Form; J. Adv. Pharm. Edu. & Res. 2014: 4(4): 440-443.

Source of Support: Nil, **Conflict of Interest:** Nil