

## A NOVEL VALIDATED STABILITY INDICATING SIMULTANEOUS ESTIMATION OF CIPROFLOXACIN AND ORNIDAZOLE BY REVERSE PHASE HIGH PRESSURE LIQUID CHROMATOGRAPHY

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### ABSTRACT

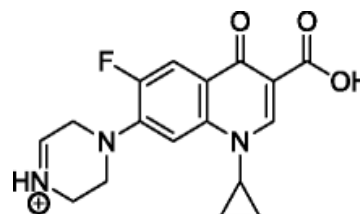
A simple, sensitive and precise RP-HPLC method for the simultaneous estimation of ciprofloxacin and ornidazole combined solid dosage form has been developed and validated. The chromatograph starting at a mobile phase of acetonitrile-water (50:50 v/v) with 0.1% of glacial acetic acid; and pH 2.8 with Orthophosphoric acid. Eluent was delivered at a flow rate of 1 ml/min. Absorbance was monitored at  $\lambda_{max}=299$  nm. The objective of current investigation was to study the degradation behaviour of ciprofloxacin and Ornidazole. The study was performed as per ICH recommended stress conditions. The solid oral dosage form was subjected to stress conditions such as oxidative, acid, base hydrolysis, heat and photolytic degradation. The method was validated for linearity, limit of detection (LOD), limit of quantification (LOQ), precision and system suitability, specificity, accuracy and robustness. The mobile phase consisted of water and acetonitrile. This validated method can be used for the routine quality control testing of ciprofloxacin and ornidazole combined solid dosage form.

### KEY WORDS

Ciprofloxacin, Ornidazole, Validation, RP-HPLC.

### INTRODUCTION

Ciprofloxacin was chemically described as 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline-carboxylic acid. Its empirical formula was  $C_{17}H_{18}FN_3O_3$  and molecular weight 331.4. Ciprofloxacin category was Broad spectrum antibiotic. Ciprofloxacin was used to treatment of Urinary tract infections, Respiratory infections, Otitis Anthrax Cervicitis. Various analytical methods have been reported for the assay of Ciprofloxacin alone and in combination with other drugs in pharmaceutical formulations. They include UV spectroscopy, HPLC, LC-MS.



**Fig. 1: The Chemical Structure of Ciprofloxacin**  
Ornidazole was chemically 1-chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl) propan-2-ol. Its molecular formula was  $C_7H_{10}ClN_3O_3$  and its molecular weight was 247.27. Ornidazole category was Anti protozoal. Ornidazole was used to treatment of Treatment of parasitic infections, Amebiasis, Giardiasis, Trichomonas vaginalis. Various analytical methods have been reported for the assay of Ornidazole alone and

in combination with other drugs in pharmaceutical formulations. They include UV spectroscopy, HPLC, LC-MS.

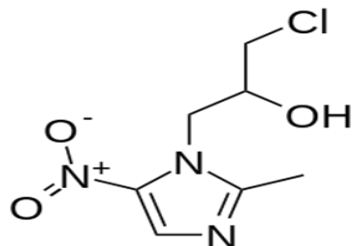


Fig. 2: The Chemical Structure of Ornidazole

## MATERIALS AND METHODS

Isocratic RP-HPLC was performed using a cyber lab chromatograph, equipped with high-pressure isocratic pump (type LC- 100), a Rheodyne model injector (sample loop 20 $\mu$ L) and LC-UV100 UV Detector (operated at 299nm) controlled by HCL PC Pentium D computer . The mobile phase consisted of water and acetonitrile. The chromatograph starting at a mobile phase of acetonitrile-water (50:50 v/v) with 0.1% of glacial acetic acid; and pH 2.8 with Orthophosphoric acid. Eluent was delivered at a flow rate of 1 ml/min. Absorbance was monitored at  $\lambda_{max}$  = 299 nm. Each tablet contains Ciprofloxacin (500mg) and Ornidazole (500mg)

Methods were available for estimation of simultaneous estimation of ciprofloxacin hydrochloride and Ornidazole in bulk and pharmaceutical dosage. A number of high performance liquid chromatographic (HPLC) methods for determination of ciprofloxacin and its metabolites in human plasma were also available.. Similarly determinations of Ornidazole by HPLC methods were also available. Pharmacopeial methods were also available for estimation of both drugs separately. No analytical method was available, which deals degradation study for combination solid dosage form. Attempts were made to develop a Liquid Chromatographic method for

the estimation of degradants and known impurities in ciprofloxacin hydrochloride and Ornidazole tablet s. Thwaspaper deals with the validation of the developed method for the accurate quantification of degradants in dosage form.

Paper was also deals with the forced degradation of ciprofloxacin and ornidazole solid dosage formulation under stress condition such as acid hydrolysis, base hydrolysis, photolytic, oxidation, and heat.

A reproducible stability indicating HPLC method was developed for the quantitative determination of ciprofloxacin and Ornidazole impurities in solid dosage formulation.

### Chemicals and reagents

Tablets, ciprofloxacin, and Ornidazole working standard and impurities were supplied by Dr. Reddy's laboratories limited, Hyderabad, India. Deionized water was prepwered using a Milli-Q plus water purification system from Millipore (Bedford, MA, USA). The HPLC grade acetonitrile, analytical grade KH<sub>2</sub>PO<sub>4</sub>, and ortho-phosphoric acid were purchaded from Merck, Mumbai, India.

### Instrumentation

Isocratic RP-HPLC was performed using a cyber lab chromatograph, equipped with high-pressure isocratic pump (type LC- 100), a Rheodyne model injector (sample loop 20 $\mu$ L) and LC-UV100 UV Detector (operated at 299nm) controlled by HCL PC Pentium D computer .The analytical column was a capcell pak C-18, 4.6 mm  $\times$  250 mm I.D (type:MG, col.no: AKAD05185, *shiseido*). The temperature was maintained at 25 $^{\circ}$ c and the data were analyzed using the cyber lab- WS-100 chromatograph workstation V4.0. Identification was based on retention times and UV-VWASspectra by comparison with commercial standards. Pci sonicator (India), eppendorf vials and borosilicate glassware were also used.

### Chromatographic conditions

The mobile phase consisted of water and acetonitrile. The chromatograph starting at a mobile phase of acetonitrile-water (50:50 v/v) with 0.1% of glacial acetic acid; and pH 2.8 with Orthophosphoric acid. Eluent was delivered at a flow rate of 1 ml/min. Absorbance was monitored at  $\lambda_{\max} = 299$  nm.

### Preparation of stock solutions

Standard stock solution of ciprofloxacin and ornidazole was prepared by dissolving 10 mg of ciprofloxacin and ornidazole in acetonitrile and water in 50:50 ratios, yielding a solution of 1mg/mL of stock solution. Series of dilutions were prepared by aliquoting 1ml from stock solution and diluted with the mobile phase to Yield 10mL of standard solutions containing 4,6,8,10,12,14  $\mu\text{g/mL}$  respectively. System suitability solution prepared by mixing (1 mg/ml) ciprofloxacin with 10  $\mu\text{g/ml}$  impurities and 1.2 mg/ml ornidazole with 12  $\mu\text{g/ml}$  impurities from above impurity stock.

### Preparation of Sample Solutions

Twenty tablets of each solid oral dosage forms were weighed and powdered in a mortar and pestle. A mass of powder equivalent to one tablet was weighed and 25 ml of mobile phase was added. The mixture was sonicated for a period of 30 minutes, agitating the mixture manually after 30 minutes and diluted with appropriate amounts of methanol and water in order to maintain a 50:50 acetonitrile:water solvent ratio in all samples. The samples were then filtered through 0.2  $\mu$  membrane filters before injection.

## RESULTS AND DISCUSSION

### Force degradation studies:

#### Acid hydrolysis

Transfer an accurately weighed amount of tablet powder equivalent to about 25 mg of ciprofloxacin to 100 ml volumetric flask,

dissolved in 50 mL of diluents then add 5 ml of 0.5 N HCl and mixed. The flask was placed at 50°C in a water bath for 5 h, After 5 h, the flask was removed and placed on bench-top to attain the laboratory temperature, add 5 ml 0.5 N NaOH to neutralized and finally made up to the volume with diluents and mixed well.

#### Base hydrolysis

Transfer an accurately weighed amount of tablet powder equivalent to about 25 mg of ciprofloxacin to 100 ml volumetric flask, dissolved in 50 ml of diluents then add 5 ml of 0.5 N NaOH and mixed. The flask was placed at 50°C in a water bath for 5 h, After 5 h, the flask was removed and placed on bench-top to attain the laboratory temperature, add 5 mL 0.5 N HCl to neutralized and finally made up to the volume with diluent and mixed well.

#### Oxidation study

Transfer an accurately weighed amount of tablet powder equivalent to about 25 mg of ciprofloxacin to 100 ml volumetric flask, dissolved in 10 ml of 3%  $\text{H}_2\text{O}_2$ . The flask was placed at 25°C in a water bath for 24 h; After 24 h, the flask was removed and finally made up to the volume with diluents and mixed well.

#### Photolytic degradation study

Transfer an accurately weighed amount of tablet powder equivalent to about 25 mg of ciprofloxacin to 100 ml volumetric flask and placed in photo stability chamber and exposed to white florescent lamp with an overall illumination of 1.2 million lux hours and near ultraviolet (UV) radiation with an overall illumination of 200  $\text{watt/m}^2/\text{h}$  at 25°C. Following removal of the flask from photo stability chamber and the drugs were finally dissolved in 50 ml diluent. The mixture was then sonicated for about 30 min, finally made up to the volume with diluents and mixed well.

### Thermal degradation study

Transfer an accurately weighed amount of tablet powder equivalent to about 25 mg of ciprofloxacin to 100 mL volumetric flask and placed in hot air oven at 80°C for 5 h. After 5 h,

the flask was removed and placed on bench-top to attain the laboratory temperature; dissolved in 50 mL diluent. The mixture was then sonicated for about 30 min, finally made up to the volume with diluents and mixed well.

**TABLE 1: Force degradation studies:**

Stress condition	Degradation time (min)	Area of peak	Degradation (%)	Active drug present after degradation (%)
Standard drug	-	168329	-	-
Acidic	3 hours	108844	27.53	73.47
Alkaline	3 hours	158335	87.88	12.46
Oxidative	3 hours	141285	70.33	29.88
Thermal	72 hours	159482	95.6	5.36
Photolytic	6 days	110083	29.89	70.10

### Method development and optimization

The main objective of the chromatographic method was to separate all degradants from both active peaks. The maximum absorption wavelength of the reference drug solution, related substances and force degradation product was 278 nm (ciprofloxacin), 316 nm (ornidazole). Initially a mobile phase composed of water and acetonitrile (50:50) (v/v) with a flow rate of 1.0 mL/min over inertsil octadecyl silane (ODS)-3V C18, 150 mm × 4.6 mm, 5 μm column was employed for separation. The EDA peak was not separate from ciprofloxacin peak. The pH of the buffer of mobile phase decreased to 2.8.

### Validation of the method

#### System suitability

System suitability parameters were measured so as to verify the system, method and column performance. Results of other system suitability parameters such as relative retention time of each impurity, tailing factor and similarity factor (between two preparations) were presented. As seen from the data, the acceptable system suitability parameters would be: relative retention time (RRT) of each impurity should

comparable, tailing factor for ciprofloxacin, ornidazole in standard solution was not more than 2.0, and resolution between all peaks should be more than 2.0. Standard chromatogram of ciprofloxacin and ornidazole were presented. Spiked chromatogram of impurity/degradation products with ciprofloxacin, tinidazole was presented.

#### Specificity

All forced degradation samples were analyzed at an initial concentration of ciprofloxacin, ornidazole with HPLC conditions mentioned in Section chromatographic conditions using PDA detector to ensure the homogeneity and purity of ciprofloxacin, ornidazole. Significant degradation of ciprofloxacin, ornidazole was observed in Heat (80°C for 5 h), photolytic UV light (200 Wh/m<sup>2</sup>), sun light (1.2 million lux hours), oxidative (3% H<sub>2</sub>O<sub>2</sub> at room temperature for 24 h), acid (0.5 N HCl at 50°C for 5 h), and base (0.5 N NaOH at 50°C for 5 h) conditions leading to the formation of impurities (% degradation should be < 0.1% > 20%).

### Precision

The % RSD for the werea of ciprofloxacin and it's known impurities (imp-EDA) and ornidazole and it's known impurities (imp-A, imp-B) in

related substances method precision was found less than 10% (should be less than 15.0%) conforming good precision of the method. The % RSD values were presented .

**TABLE2: RESULTS OF INTER-DAY AND INTRA-DAY PRECISION FOR CIPROFLOXACIN**

Ciprofloxacin	Intra Day	Inter Day
<b>Injection 1</b>	522355	525914
<b>Injection 2</b>	520851	527346
<b>Injection 3</b>	532356	525765
<b>Average</b>	525765	522355
<b>S.D</b>	45206.4	45321.7
<b>R.S.D</b>	0.86	0.84

**TABLE3: RESULTS OF INTER-DAY AND INTRA-DAY PRECISION FOR ORNIDAZOLE**

Ornidazole	Intra -day	Inter-Day
<b>Injection 1</b>	274245	279357
<b>Injection 2</b>	276275	277848
<b>Injection 3</b>	279767	277498
<b>Average</b>	276264	277538
<b>S.D</b>	22806.4	<b>22532.2</b>
<b>R.S.D</b>	0.82	<b>0.84</b>

### LOD and LOQ

The determination of limit of detection and limit of detection (LOD) of all impurities namely ciprofloxacin and it's known impurities (imp-EDA) and ornidazole and its known impurities (imp-A, imp-B) were reported in. The precision at the LOQ concentrations for ciprofloxacin and its known impurities (imp-EDA) and ornidazole and its known impurities (imp-A, imp-B) were found below 10% (should be less than 15.0%). Limit of detection, LOQ of all impurities values presented.

### Linearity

The result shows that an excellent correlation existed between the peak werea and concentration of the analyte. Linear calibration plot for the related substance method was obtained over the calibration ranges tested, i.e., LOQ to 200% for impurity (ciprofloxacin and its known impurities (imp-EDA) and ornidazole and its known impurities (imp-A, imp-B)). The correlation coefficient obtained was greater than 0.997. The above result show that an excellent correlation existed between the peak werea and the concentration. The % bias also calculated for all related compounds and main analytes and found less than 5%.

**TABLE3: LINEARITY OF CIPROFLOXACIN AND ORNIDAZOLE**

S.No.	Concentration( $\mu\text{g/ml}$ )	Peak area Of Ciprofloxacin	Peak area of Ornidazole
1.	0.0	0.0	0.0
2.	4	55490.1	21213.4
3.	6	86643.7	64278.1
4.	8	127961.5	90207.6
5.	10	175052.8	135182.5
6.	12	222156.2	186571.5
7.	14	254186.0	212458.1

**Accuracy**

The percentage recovery of ciprofloxacin and its known impurity and ornidazole and its known impurities in tablet varied from 90% to 110% at LOQ, 50%, 100%, 125% and 150% levels of target 0.2% level of respective target

concentrations. The LC chromatogram of spiked sample at 0.2% level of all three impurities in the sample solution was shown. Recovery values for impurities were presented (% recovery should be in between 90% and 110%).

**TABLE 4: ACCURACY OF CIPROFLOXACIN:**

Sample id	Concentration mcg	Percentage Recovery	Mean percentage recovery	Standard deviation	Relative standard deviation
1	50%	101.11			
2	50%	101.11	101.36	0.433013	0.427203
3	50%	101.86			
4	100%	100.306			
5	100%	100.93	101.0553	0.217084	0.214817
6	100%	100.93			
7	150%	98.13			
8	150%	98.88	98.50333	0.375011	0.380709
9	150%	98.5			

**TABLE 5: ACCURACY OF ORNIDAZOLE:**

Sample id	Concentration mcg	Percentage Recovery	Mean percentage recovery	Standard deviation	Relative standard deviation
1	50%	98.04			
2	50%	98.04	98.39667	0.617765	0.627831
3	50%	99.11			
4	100%	99.46			
5	100%	99.46	99.93333	0.819837	0.820384
6	100%	100.88			
7	150%	99.7			
8	150%	99.46	99.54	0.138564	0.139204
9	150%	99.46			

### Robustness

In all deliberate varied chromatographic conditions (flow rate, column temperature and pH of mobile phase buffer); the resolution between critical pairs was greater than 2.0, illustrating the robustness of the method.

### Stability in solution

No significant changes were observed in the content of impurities namely ciprofloxacin and its known impurities (imp-EDA) and ornidazole and its known impurities (imp-A, imp-B) during solution stability experiments when performed using the related substances method. The solution stability experiment data confirms that the sample solutions used during the related substances determination were stable for 24 h.

### CONCLUSION

A new sensitive method was developed and optimized; the following parameters were validated according to ICH guidelines:

1. Mobile phase-Acetonitrile: water 70:30(Ortho phosphoric acid)
2. Limit of detection (LOD) - 400ng.
3. Limit of quantification (LOQ) - 1400ng.
4. Linearity-4-14µg/ml.
5. Precision, accuracy & robustness were performed.

The gradient RP-HPLC method developed for ciprofloxacin and ornidazole and related substances in solid pharmaceutical dosage forms was found precise, accurate, linear, robust, rugged, and specific. Satisfactory results were obtained from validation of the method. Hence, the method was stability-indicating and can be used for routine analysis of production samples and to check the stability of samples.

### REFERENCES

1. Kluwer W; Remington – The science and practice of pharmacy; 21<sup>st</sup> edition; 2005; Vol(1); pp:599-632.

2. Settle FA; Handbook of instrumental techniques for analytical chemistry; Pearson Education; 2004; pp:147-154.
3. Chatwal GR, Anand SK; Instrumental methods of chemical analysis; Himalaya Publishing House; 2004; pp:2.567-2.585
4. Watson DG; Pharmaceutical Analysis; Elsevier churchill livingstone publications; 2<sup>nd</sup> edition; 2005; pp:87-108.
5. Sethi PD; High performance liquid chromatography, quantitative analysis of pharmaceutical formulations; CBS publications; 1<sup>st</sup> edition; 2001; pp:1-212.
6. Suresh R, Anarthanan VJ, Manavalan R, Valliappan K; Aspects of validation in HPLC method development for pharmaceutical analysis- comparison of validation requirements by FDA, USP and ICH; International Journal of Pharmaceutical Sciences and Research; 2010; 1 (12)pp: 123-132.
7. Betz JM, Brown PN, Roman MC; Accuracy, precision, and reliability of chemical measurements in natural products research; Fitoterapia; 2011;82:pp: 44–52.
8. International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, Topic Q2 (R1): Validation of analytical procedures: Text and Methodology, Geneva, 2005.
9. Virlichie JL, Ayache A; A ruggedness test and its application for hplc validation; S. T. P. Pharma Pratiques; 1995; pp: 37.
10. Braun RD; Introduction to instrumental analysis; Pharma Book Syndicate; 2006; pp:839-868.
11. Khandpur RS; Hand book of analytical instruments; Mc Graw-Hill publishers; 2005; pp:383-401.
12. Snyder LR, Kirkland JJ, Glajch JL; Practical HPLC method development; Wiley interscience publications; 2<sup>nd</sup> edition; 1997; pp:21-174.
13. Willard HH, Merrit LL (Jr), Dean JA, Settle FA (Jr). Instrumental methods of analysis. 6th ed. New Delhi: CBS Publishers And Distributors; 1986. pp. 318-20.
14. Skoog DA, Holler FJ, Crouch SR. Instrumental analysis. 3rd Ed. New Delhi: Cengage Learning India Pvt Ltd; 2009. pp. 13-6, 158.

15. Connors KA. A textbook of pharmaceutical analysis. 3rd ed. Delhi: Wiley Intersciences Inc; 1994. p p. 216-24, 581-604.
16. Sharma YR. Elementary organic spectroscopy. 4th ed. New Delhi: S Chand & Company Ltd; 2007. pp. 9-13.
17. Mendham J, Denny RC, Thomas M Vogel's text book of quantitative practical organic chemistry. 5th ed. Singapore: Longman Singapore Publishers Pvt Ltd; 2004. pp. 577-578.
18. Skoog DA, West DM, Holler FJ and Crouch SR. Fundamentals of analytical chemistry. 8th ed. Singapore: Thomson Brooks; pp. 160-7.
19. S\*Manoranjan ,G.Venkateshwarlu, Mahesh.S, Stability indicating UVspectrophotometer determination of ciprofloxacin in pharmaceutical dosage form International Journal Of Pharmaceutical Chemistry Research ,Volume 2 Issue 1 pp 207-17.
20. Wu SS, Chein CY and Wen YH: Analysis of ciprofloxacin by a simple high performance liquid chromatography method. Journal of Chromatographic Science 2008; 46:pp 490-495.
21. Witte BD, Dewulf J, Demeestere K, Ruyck MD and Langenhove HV: Critical points in the analysis of ciprofloxacin by high-performance liquid chromatography. Journal of Chromatography 2007; pp 126-130.
22. Fratini L and Schapoval ES: Ciprofloxacin determination by visible light spectrophotometry using iron(III)nitrate. International Journal of Pharmaceutics 1996; pp127, 279-282.
23. Diao YH: Determination of ciprofloxacin hydrochloride with UV method and quality investigation of its tablets. Chinese Journal of Hospital Pharmacy 1994; 14: pp212-214.
24. Krol GJ, Beck GW and Benham T: HPLC analysis of ciprofloxacin and ciprofloxacin metabolites in body fluids. Journal of Pharmaceutical and Biomedical Analysis 1995; 14, pp181-190.
25. Singh BK, Parwate DV, Srivastava S and Shukla SK: High performance thin-layer chromatographic selective and stability indicating method for assay of ciprofloxacin in pharmaceuticals. Der Pharma Chemica 2010; 2(4):pp 178-188.



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