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

Kumara Swamy Damerakonda[*1] & M.Hima Bindu[2]

Department of Pharmaceutical Analysis, Vaagdevi College of pharmacy, kishan pura, Hanamkonda, Warangal, Thelengana state-506001, India.

***Corresponding Author Email: dks.july12@gmail.com**

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 λ_{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.

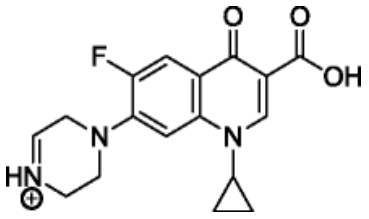

Fig. 1: The Chemical Structure of Ciprofloxacin

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 was247.27. 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.

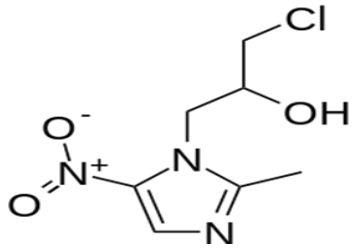

Fig. 2: The Chemical Structure of Ornidazole

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 20µL) 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 λmax = 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 wasavailable, 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_2PO_4, 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 20µL) 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 × 250 mm I.D (type:MG, col.no: AKAD05185, *shiseido*). The temperature was maintained at 25°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 λ_{max} = 299 nm.

Preparation of stock solutions

Standard stock solution of ciprofloxacin and ornidazole was prepwered 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 prepwered 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 µg/mL respectively.System suitability solution prepwered by mixing (1 mg/ml) ciprofloxacin with 10 µg/ml impurities and 1.2 mg/ml ornidazole with 12 µg/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 µ 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% H_2O_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 camber 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 watt/m^2/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 was278 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 thwasdata, the acceptable system suitability parameters would be: relative retention time (RRT) of each impurity should comparable, tailing factor for ciprofloxacin, ornidazole in standard solution wasnot more than 2.0, and resolution between all peaks should be more than 2.0 Presented . Standard chromatogram of ciprofloxacin and ornidazole were presented . Spiked chromatogram of impurity/degradation products with ciprofloxacin, tinidazole waspresented .

Specificity

All forced degradation samples were analyzed at an initial concentration of ciprofloxacin,orinidazole 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^2), sun light (1.2 million lux hours), oxidative (3% H_2O_2 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
Injection 1	522355	525914
Injection 2	520851	527346
Injection 3	532356	525765
Average	525765	522355
S.D	45206.4	45321.7
R.S.D	0.86	0.84

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

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

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(µg/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 it's 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 wasshown . 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.

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***Corresponding Author:**
dks.july12@gmail.com