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Method Development and Validation for Ciprofloxacin and Ornidazole in Its Bulk and Combined Dosage Forms by RP–HPLC

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ABSTRACT

The healthcare expenditure is continuously growing at an unprecedented and unsustainable rate. With the shift to value-based care, healthcare organizations are expected to provide consistent high-quality, safe care while reducing healthcare costs. As reimbursements shrink, healthcare organization leadership and clinical providers must identify opportunities to minimize unnecessary practice variation while providing high-value healthcare. In recent years, the therapeutic landscape has changed with the proliferation of specialty drugs, which are used in the management of an array of medical conditions, including cancers, chronic infections, autoimmune disorders, transplantation, and bleeding disorders. Loosely defined based on their high costs, the need for special handling protocols, and close patient monitoring, specialty drugs are projected to account for 50% of the total medical expenditure by 2019. The biologic agents, which are produced or derived from a living organism, are the most rapidly growing class of specialty drugs, and hold promise to revolutionize the management of a range of chronic medical conditions. The challenge, however, is reconciling the potential therapeutic benefit with the high cost of these agents. Specialty drugs contribute significantly to the inpatient diagnosis-related group payment system, often with unproved benefits over less-expensive treatments.

Keywords: Healthcare expenditure, reimbursements shrink, high cost, protocol, biologic agents, medical conditions.

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1. Introduction

Ciprofloxacin is a broad-spectrum antimicrobial carboxyfluoroquinoline. The bactericidal action of ciprofloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial DNA replication,

transcription, repair, strand supercoiling repair, and recombination.

IUPAC Name: 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid

Chemical formula : $C_{17}H_{18}FN_3O_3$

Molecular weight : 331.3415

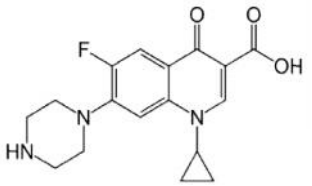

Figure 1

Ornidazole has been used in trials studying the prevention of Elective Colorectal Surgery.

IUPAC Name: 1-chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl) propan-2-ol

Chemical formula: $C_7H_{10}ClN_3O_3$

Molecular weight: 219.63

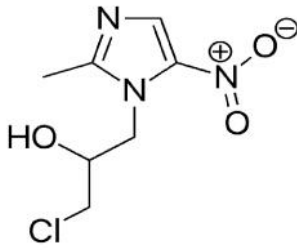

Figure 2

2. Methodology

Method development:

Method development for simultaneous estimation of Ciprofloxacin and Ornidazole in Pharmaceutical dosage forms includes the following steps:

- Selection of detection wavelength (λmax)
- Selection of column
- Selection of mobile phase
- Selection of flow rate
- Preparations and procedures

Selection of Detection wavelength:

10mg of Ciprofloxacin and Ornidazole was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Ciprofloxacin and Ornidazole. The isobestic point was taken as detection wavelength.

Selection of column:

Column is selected based on solubility, polarity and chemical differences among Analytes [Column: Inertsil C18 (4.6 x 250mm, 5µm, Make: Waters)]

Selection of mobile phase:

Phosphate buffer (0.05M) pH 4.6: ACN (30:70%v/v) has been selected as mobile phase. Buffer pH should be between 2 to 8. If the buffer pH is below 2 siloxane linkages are cleaved. If the buffer pH is above 8 dissolution of silica takes place. pH controls the elution properties by controlling the ionization characteristics. It also decreases the retention and improves separation. Good Response, Area, Tailing factor, Resolution will be achieved.

4. Selection of flow rate:

Flow rate selected was 1ml/min

Flow rate is selected based on

1. Retention time
2. Column back pressure
3. Peak symmetry
4. Separation of impurities
5. Preparations and procedures:

Preparation of Phosphate buffer :(PH: 4.6):

Weighed 6.8 grams of KH2PO4 was taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water, adjusted the pH to 4.6 with ortho phosphoric acid.

Preparation of mobile phase:

A mixture of pH 4.6 Phosphate buffer 300 mL (30%), 700 mL of ACN (70%) are taken and degassed in ultrasonic water bath for 5 minutes. Then this solution is filtered through 0.45 µ filter under vacuum filtration.

Diluant Preparation:

Mobile phase is used as Diluant.

Preparation of the individual Ciprofloxacin standard preparation:

10mg of Ciprofloxacin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluant (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluant.

Preparation of the individual Ornidazole standard preparation:

10mg of Ornidazole working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluant. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluant.

Preparation of Sample Solution :(Tablet)

Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to 10 mg of Ornidazole and Ciprofloxacin (marketed formulation) sample into a 10mL clean dry volumetric flask and about 7mL of Diluents is added and sonicated to dissolve it completely and made volume upto the mark with the same solvent. (Stock solution) Further 3 ml of above stock solution was pipetted into a10ml volumetric flask and diluted upto the mark with diluant.

Procedure:

20µL of the standard, sample are injected into the chromatographic system and the areas for Ornidazole and Ciprofloxacin peaks are measured and the %Assay are calculated by using the formulae.

System Suitability:

- Tailing factor for the peaks due to Ornidazole and Ciprofloxacin in Standard solution should not be more than 2.0.
- Theoretical plates for the Ornidazole and Ciprofloxacin peaks in Standard solution should not be less than 2000

System suitability:

5 mg of Ciprofloxacin and 500mg of Ornidazole working standard was accurately weighed and transferred into a 100ml clean dry volumetric flask and add about 20ml of diluant and sonicated to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further 10 ml of Ciprofloxacin and Ornidazole was pipetted out from the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluant.

3. Results and Discussion

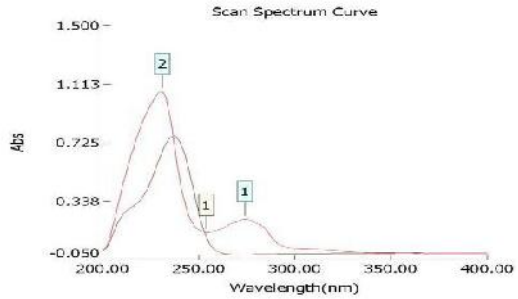

Figure 3: Overlay spectrum of Ciprofloxacin and Ornidazole

Trial-1: Chromatographic conditions

Column : Agilent C18 (4.6*150mm) 5µm
Mobile phase ratio: Water: Methanol (40:60%v/v)
Detection wavelength: 255nm
Flow rate: 1ml/min
Injection volume: 10µl
Column temperature: Ambient
Auto sampler temperature: Ambient

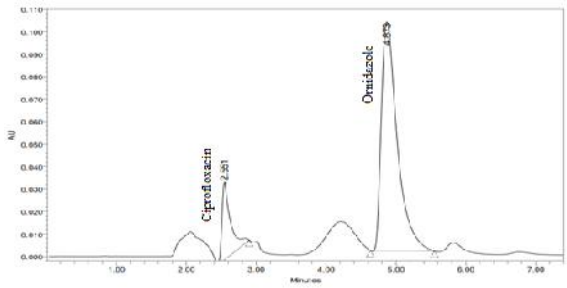

Figure 4: Chromatogram of Trial-1

Trial-2: Chromatographic conditions:

Column: Thermosil C18 (4.6*150mm) 5µm
Mobile phase ratio: Water: Methanol (40:60%v/v)
Detection Wavelength: 255nm
Flow rate: 1ml/min
Injection volume: 10µl
Column temperature: 40°
Auto sampler temperature: Ambient

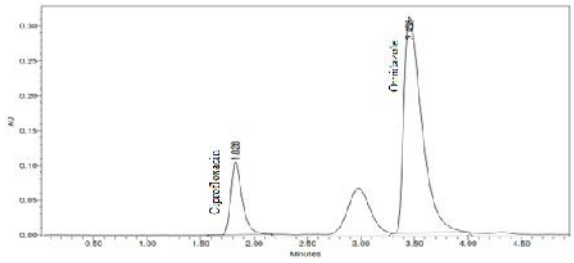

Figure 5: Chromatogram of Trial-2

Method Optimization

Chromatographic conditions:
Column: Inertsil C18 5µm (4.6*250mm)
Mobile phase ratio: Phosphate buffer (0.05M) pH 4.6: ACN (30:70%v/v)
Detection wavelength: 255nm
Flow rate: 1ml/min
Injection volume: 20µl
Column temperature: Ambient

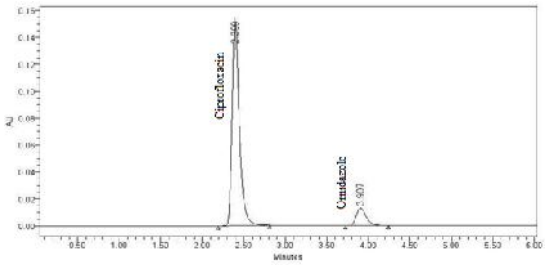

Figure 6: Optimized chromatogram

Observation:

The chromatogram is perfect with clear separation of components. The peak symmetry and system suitability parameters are within the limits. Hence this method is chosen as optimized one.

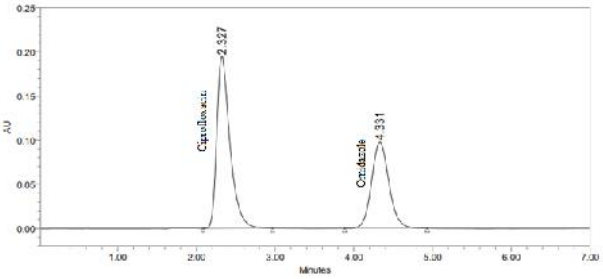

Figure 7: Chromatogram showing sample injection-1

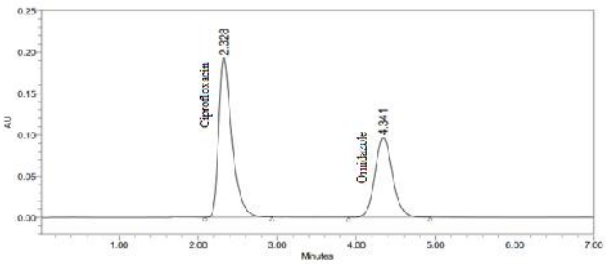

Figure 8: Chromatogram showing sample injection-2

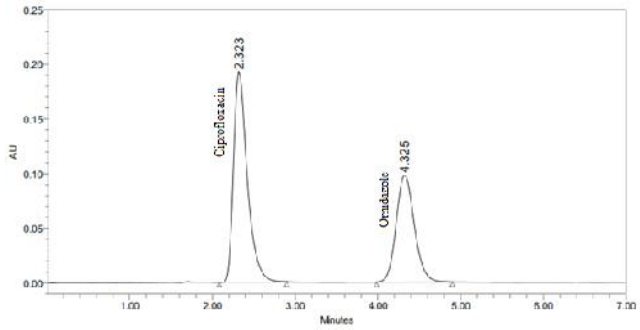

Figure 9: Chromatogram showing standard injection-3

Assay calculations

$$Assay\ \% = \frac{sample\ area}{Standard\ area} \times \frac{dilution\ sample}{dilution\ of\ standard} \times \frac{P}{100} \times \frac{Avg.wt}{Lc} \times 100$$

$$\frac{776673.9 \times 10 \times 0.5 \times 100 \times 10 \times 99.8 \times 0.668 \times 100}{771716.1 \times 10 \times 10 \times 458 \times 0.33 \times 100 \times 500}$$

Ornidazole:

Wt of 10 tablets: 668 g

Avgas wt: 0.668 g

Assay % = 101.4

Ciprofloxacin:

Wt of 10 tablets 458 g.

Avgas wt: 0.458 g.

Assay% =100.7%

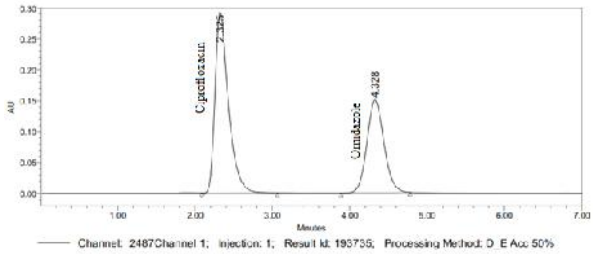

Figure 10: Accuracy chromatogram 50%injection-1

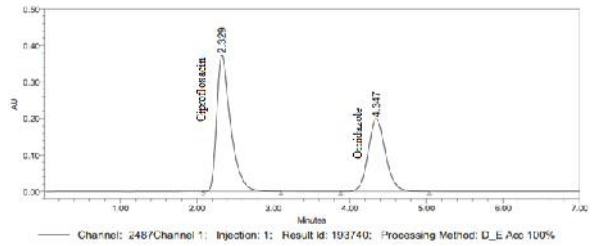

Figure 11: Accuracy chromatogram 100%injection-1

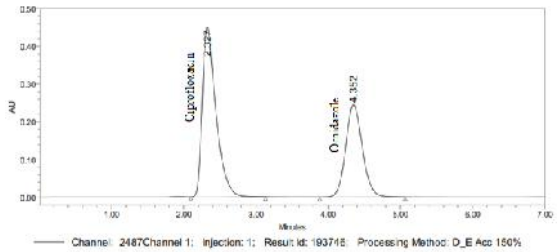

Figure 12: Accuracy chromatogram 150%injection-1

Table 1: Repeatability results of Ciprofloxacin & Ornidazole

Injection	Area
Injection-1	1501417
Injection-2	1486940
Injection-3	1490656
Injection-4	1487329
Injection-5	1490384
Average	1491345
Standard Deviation	5881.4
%RSD	0.39

Injection	Area
Injection-1	2235319
Injection-2	2240678
Injection-3	2249490
Injection-4	2245822
Injection-5	2251694
Average	2244601
Standard Deviation	6656.8
%RSD	0.32

Acceptance Criteria:

The % RSD for the area of five standard injections results should not be more than 2%. The Method precision study was performed for the %RSD of Ciprofloxacin and Ornidazole was found to be 0.3 and 0.3 (NMT 2).

Table 7.9 Ruggedness results of Ornidazole and Ciprofloxacin

Injection	Area
Injection-1	2194758
Injection-2	2195700
Injection-3	2196191
Injection-4	2195326
Injection-5	2200951
Average	2196585
Standard Deviation	2496.0
%RSD	0.11

Injection	Area
Injection-1	1456296
Injection-2	1457422
Injection-3	1456513
Injection-4	1454579
Injection-5	1451483
Average	1455259
Standard Deviation	2347.6
%RSD	0.16

Acceptance Criteria: The % RSD for the area of five standard injections results should not be more than 2%. The intermediate precision was performed for %RSD of Ciprofloxacin and Ornidazole was found to be 0.1 and 0.1 respectively (NMT 2).

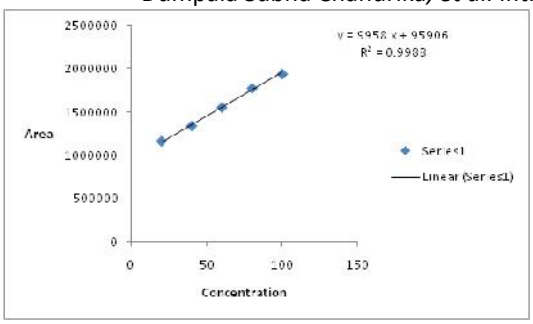

Figure 13: Calibration curve of Ornidazole

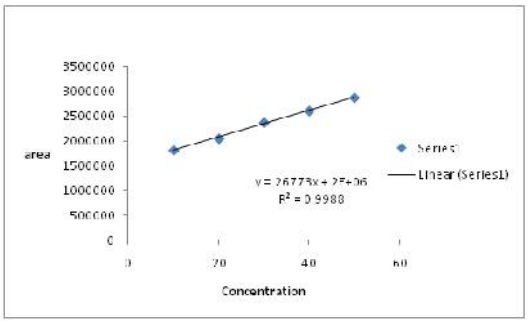

Figure 14: Calibration curve of Ciprofloxacin

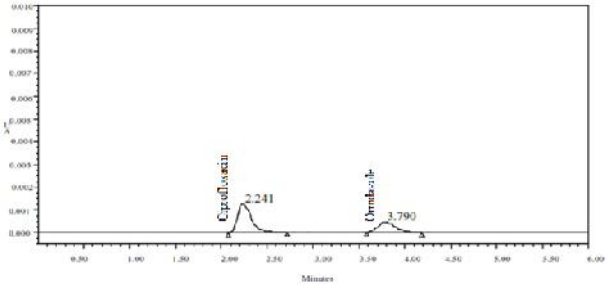

Figure 15: Results of LOD

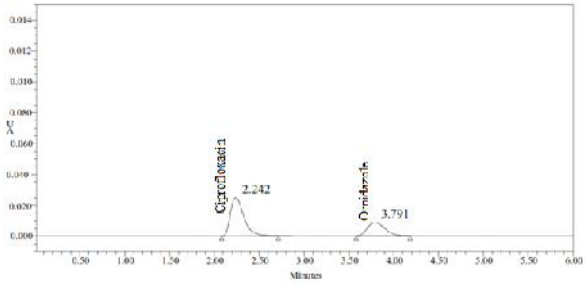

Figure 16: Results of LOQ

Ornidazole

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank: 41 µV Signal Obtained from LOQ solution: 412µV

S/N = 412/41 = 10.0

Acceptance Criteria:

S/N Ratio value shall be 10 for LOQ solution.

Ciprofloxacin

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank: 41 µV Signal Obtained from LOQ solution: 405µV

S/N = 405/41 = 9.87

4. Conclusion

The analytical method was validated according to ICH guidelines (ICH,Q2(R1)). The linearity study for Ciprofloxacin and Ornidazole was found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 100% and 100.5%, %RSD for repeatability was 0.2 and 0.4, % RSD for intermediate precision was 0.5 and 0.1 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.95 and 3.04, and LOQ value was 9.87 and 10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Ciprofloxacin and Ornidazole in API and Pharmaceutical dosage form.

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