# Comparative & Combine HPLC Method of Development of Drugs and Validation of Cefixime & Ornidazole

**Suman Sharma[1]* Dr. Satyavir[2]**

[1] Research Scholar, Department of Chemistry, OPJS University, Rajasthan

[2] Associate Professor, Department of Chemistry, OPJS University, Rajasthan

***Abstract – Cefixime is an oral third-generation cephalosporin antibiotic. It is used to treat gonorrhea, tonsillitis and pharyngitis. The chromatography of the present invention is used to determine Cefixime trihydrate and Ornidazole in bulk drugs and pharmaceutical dosage forms. Separation and quantification were achieved on ACEC 18, 5µm,150 x 4.6 mm i. d. column. The Cefixime trihydrate and Ornidazole were exposed to acidic, basic, oxidative, neutral, thermal, and photolytic stress conditions, and stress samples were analyzed. The linearity of the Cefixime trihydrate concentration in the range of 10 - 60 µg / mL was good, the correlation coefficient was 0.998, the Ornidazole correlation coefficient was 0.998, 25 - 150 µg / mL, and the correlation coefficient was 0.997. The intraday precision and accuracy of both analytes were less than 2% RSD.***

***Keywords: Cefixime Trihydrate, Ornidazole, Stability Indicating, Analytes.***

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## 1.1 INTRODUCTION TO DRUG CEFIXIME

The Cefixime is an antibiotic that can be used to treat many bacterial infections. This includes otitis media, pharyngitis, pneumonia, urinary tract infections, gonorrhea and Lyme disease(Choragudi, et. al., 2015). For gonorrhea, usually only one dose is required (Adam, et. al., 2005). It is taken with his mouth. Common side effects include diarrhea, abdominal pain, and nausea (Choragudi, et. al., 2015). It is not recommended to use in people with a history of severe penicillin allergies (Adam, et. al., 2005). It seems relatively safe during pregnancy (Rutgeerts, et. al., 2015). It is the third generation of cephalosporins. It works by destroying the cell wall of bacteria causing its death.

### 1.1.1 Basic Chemical Data Method

The Cefixime is chemically (6R,7R)-7-{[2-(2-amino-1,3-thiazol-4-yl)-2-(carboxy methoxyimino)acetyl]amino}-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid showing in Figure 1. Its molecular formula is C16H15N5O7S2 cephalosporin having molecular weight 453.45 gm/mole. Cefixime is an oral third generation antibiotic. It is used to treat gonorrhea, tonsilitis, and pharyngitis.

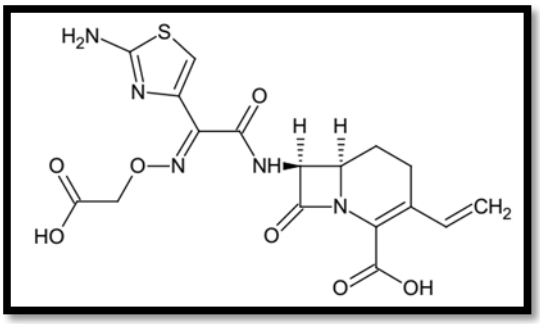


**Figure 1:** Showing (6R,7R)-7-{[2-(2-amino-1,3-thiazol-4-yl)-2-(carboxy methoxyimino) acetyl]amino}-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid

## 1.2 INTRODUCTION TO DRUG ORNIDAZOLE

The Ornidazole is a drug that cures some protozoan infections. It is used by the poultry industry. It has been investigated for use in Crohn's disease after bowel resection (Rutgeerts, et. al., 2015).

### 1.2.1 Basic Chemical Data Method

The Ornidazole is chemically 1-chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol Figure 2. Its molecular formula is C7H10ClN3O3 protozoan

having molecular weight 219.63 gm/mole. Ornidazole is a drug that cures some infections. It is used by the poultry industry .It has been investigated for use in Crohn's disease after bowel resection.

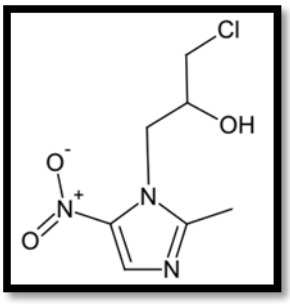


**Figure: 2** Showing 1-chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol

### 1.2.2 Mechanism of Action

The Ornidazole is a 5-nitroimidazole derivative active against protozoa and anaerobic bacteria. It is converted to reduction products that interact with DNA to cause destruction of helical DNA structure and strand leading to a protein synthesis inhibition and cell death in susceptible organisms.

## 1.3 REVIEW OF LITERATURE CEFIXIME & ORNIDAZOLE

The literature review of Cefixime that various methods of analysis, it was determined that the drug in pharmaceutical formulations and in various biological fluids. The literature review and analysis of the Cefixime and ornidazole are the following:

**According to R. Jain, V. K. Gupta, N. Jadon** and **K. Radhapyari** method has been developed, the voltammetric determination of Cefixime in the pharmaceutical and biological fluids. Electro reduction and adsorption of Cefixime in the phosphate buffer of cyclic voltammetry (CV), the Differential Pulse cathodic adsorption stripping voltammetry (DPCADSV),and Square Wave cathode adsorption stripping (SWCADSV voltammetry), in the hanging mercury drop electrode (HMDE). The cathode adsorption stripping voltammetric procedures, tracking the determination bulk drugs and pharmaceutical formulations in the urine (Jain, et. al., 2010).

**According to R. K. Nanda, J. Gaikwad** and **A. Prakash** method has been developed to estimate the Cefixime and ornidazole in its pharmaceutical dosage forms, and spectrophotometric methods. Two accurate, precise, quick and cost-effective methods to estimate Cefixime and ornidazole in tablet dosage form.

The first method is the first order of derivative financial instruments of the spectrum, quantitation of the selected wavelength of 311.5 Nm of the Cefixime (zero-crossing of ornidazole) and 290.0 nm of ornidazole (zero-crossing of the Cefixime). Second method was area under curve method; area under curve in the range of 295.0-285.0 nm (for Cefixime) and 317.0-307.0 nm (for ornidazole) were selected for the analysis (Nanda, et. al., 2009).

**According to R. K. Nanda, J. Gaikwad,** and **A. Prakash** have development of the method, and spectrophotometric estimation, in tablet and ornidazole Cefixime dosage forms. Two precise, accurate, quick and cost-effective method to estimate Cefixime and ornidazole in tablet dosage form. The first method is based on the simultaneous equations and wavelength selection for analysis is 290.0 Nm (λ max of Cefixime) and 312.0 Nm (λ max, ornidazole) on each of the methanol. Second method was Q-analysis method based on absorbance ratio at 2 selected wavelengths 303.0 nm (iso-absorptive point) and 312.0 nm (λmax of Ornidazole) (Nanda, et. al., 2009).

**According to N. Sreekanth, Ch. B. Rao, P. Ramalingam, P. Seetharamaiah** and **S. Ganapaty** have method has been developed, and spectrophotometric estimation and validation of Cefixime and ornidazole volume and drug dosage form of the absorption rate analysis methods. A simple method developed the ultraviolet spectrophotometric determination of Cefixime and ornidazole in bulk and its pharmaceutical formulations of the absorption rate.

In this method, the solution absorbances were measured at two wavelengths, one Wavelength peak of one of the component and the rest of the absorptivity of the wavelength is equal to that of two parts. Ornidazole Cefixime and participate in maximum absorption peak wavelength of 289 nm and 310 nm, respectively, in the methanol (Sreekanth, et. al., 2015).

## 1.4 THE OBJECTIVES OF CURRENT WORK

At the same time as per discussion in the literature review, determination method of Cefixime and ornidazole but most of the methods are applicable to alone Cefixime or ornidazole in pharmaceutical dosage form or in biological fluids. The Voltammetric determination of spectrophotometry, Fluorescent, thin layer chromatography, the first derivative and spectrophotometric and HPLC methods. Only the three methods have been reported, while at the same time determine a Cefixime and ornidazole.

It is the ultraviolet spectrophotometric determination, which is the capability to identify and ornidazole Cefixime dose in the form of combination. So far, our knowledge is a matter of concern, no HPLC analytical methods for the determination of the combine ornidazole Cefixime dosage forms have

been published. Previously published methods are not directly applicable to this issue that requires more investigation method development and validation.

Therefore, in the present study focused on the development of a validated simple, Precise and accurate HPLC method.

## 1.5 EXPERIMENTAL METHOD

### 1. Materials

The Cefixime and Ornidazole standard of was provided by Nectar Drugs Ltd. (India). Cefixime and Ornidazole tablets containing 200mg Cefixime and 500mg ornidazole (mahasaif-OZ) and the inactive ingredient used in drug matrix were obtained from market. HPLC grade acetonitrile, methanol and water were obtained from Spectrochem
Private Ltd., Mumbai (India).

### 2. Instrumentation

The chromatographic system used to perform development and validation of this assay method was comprised of a LC-10ATvp binary pump, a SPD-M10Avp photo-diode array detector and a rheodyne manual injector model 7725i with 20µl loop (Shimadzu, Kyoto, Japan) connected to a multi-instrument data acquisition and data processing system (Class-*VP* 6.13 SP2, Shimadzu).

### 3. Mobile phase preparation

The mobile phase consisted of Water: Acetonitrile: Methanol (50:25:25, v/v).Mobile phase was filtered through a 0.45µm nylon membrane (Millipore Private Ltd. Bangalore, India) and degassed in an ultrasonic bath (Spincotech Private Ltd., Mumbai).

### 4. Diluents Preparation

Methanol used as diluents.

### 5. Standard Preparation

The Cefixime standard stock solution containing 2000µg/ml was prepared in a 100 ml volumetric flask by dissolving 200.00 mg of Cefixime along with then diluted to volume with diluent. Further take 2 ml of this stock solution in 50 ml volumetric flask and make up to mark with diluent (this standard solution of 80µg/ml). The Ornidazole standard stock solution containing 5000µg/ml was prepared in a 100 ml volumetric flask by dissolving 500.00 mg of ornidazole and then diluted to volume with diluent. Further take 2 ml of this stock solution in 50 ml volumetric flask and make up to mark with diluent (this standard solution of 200µg/ml).

### 6. Test Preparation

Twenty tablets were weighed and the average weight of tablet was determined. From these, five tablets were weighed and transfer into a 500 ml volumetric flask. In relation to 50 ml of diluent was added and sonicated for a minimum 30 minute with irregular shaking. After that content was brought back to room temperature and diluted to volume with diluent. The sample was filtered through 0.45µm nylon syringe filter. Further take 2 ml of this stock solution in 50 ml of volumetric flask and make up to mark with diluent. The concentration obtained was 80 µg/ml of Cefixime and 200 µg/ml of ornidazole

### 7. Chromatographic Conditions

The Chromatographic analysis was performed on an Aqurasil SS (150mm × 4.6mm i.d., 5 µm particle size) column. The flow rate of the mobile phase was adjusted to 0.6 ml/min and the injection volume was 20 µl. The Detection was performed at 304nm.

## 1.6 RESULT AND DISCUSSION OF WORKS

### 1.6.1 Development and Optimization of the HPLC Method

In the present work, an analytical method based on LC using UV detection was developed and validated for assay determination of Cefixime and ornidazole in tablet formulation. The analytical conditions were selected, keeping in mind the different chemical nature of Cefixime and Ornidazole.

The feature selection has been done on the basis of backpressure, resolution, peak shape, theoretical plates and day-to-day reproducibility of the retention time and resolution between Cefixime and ornidazole peak. After evaluating all these factors, an Aqurasil SS (150mm × 4.6mm i.d., 5 µm particle size) column was found to be giving satisfactory results.

The collection of water, acetonitrile, methanol based on chemical structure of both the drugs. These solvent compositions were found suitable for solubility, resolution, stability, theoretical plates and peak shape of both components. Best results were obtained with Water: Acetonitrile: Methanol solution improved the peak shape of Cefixime and ornidazole. Finally, by fixing mobile phase composition consisting of a mixture of Water, Acetonitrile, and Methanol (50:25:25, v/v).

Optimize mobile phase proportion was provide good resolution between Cefixime and Ornidazole. Wavelength selection and PDA scan graph are given Figure 3 and 4 respectively. Figure 5 and 6

represents the chromatograms of standard and test preparation respectively.

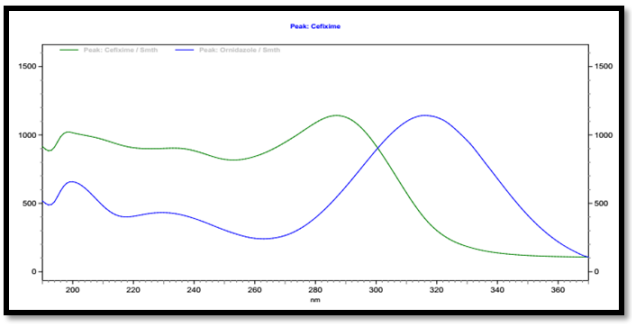


**Figure 3:** Showing Wavelength Scan Overlay of Standard Preparation

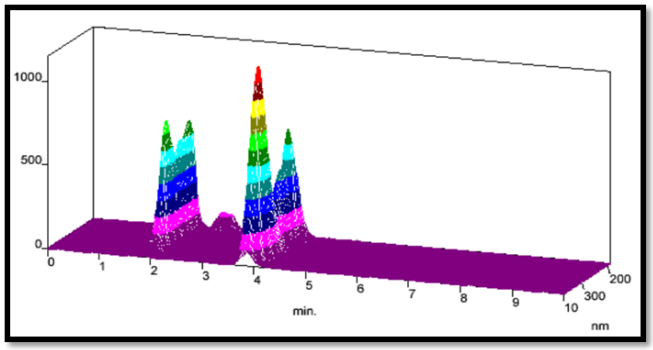


**Figure 4:** Showing PDA Scans of Standard Preparation

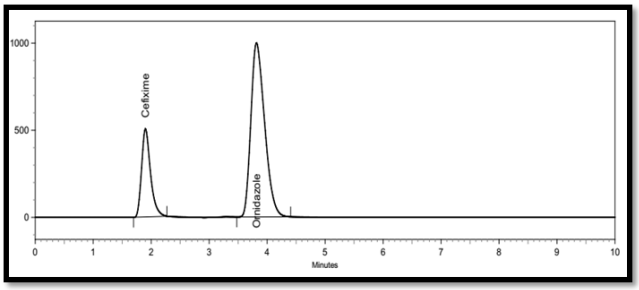


**Figure 5:** Showing Chromatogram of Standard Preparation

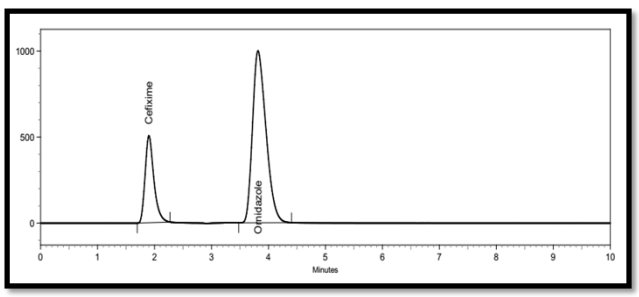


**Figure 6:** Showing Chromatogram of Test Preparation

### 1.6.2 Validation Method

### 1. Specificity

In an experiment, the model's specific requirements, it can be shown that this program would not be affected by the presence of impurities or excipients. In practice, this is done with the peak of drug substances or products with the appropriate level of impurities or excipients and proves that the results will not be affected by the presence of these irrelevant materials. There should not be any interference of the thinner, placebo's retention time of drug substances.

### 2. Linearity

For linearity seven points calibration curve were obtained in a concentration range from 0.032-0.128 mg/ml for Cefixime and 0.08-0.32 mg/ml for Ornidazole. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation for Cefixime was y = 7E+07x + 112940 with correlation coefficient 0.9992 (Figure 7) and for Ornidazole was y = 8E+07x + 73653 with correlation coefficient 0.9999 (Figure 8). Where x is the concentration in mg/ml and y is the peak area in absorbance unit.

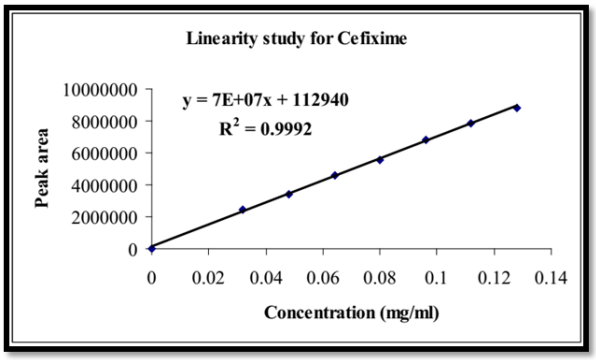


**Figure 7:** Showing Linearity curve for ***Cefixime***

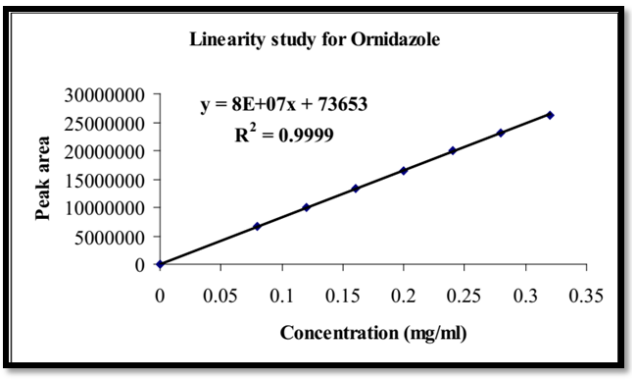


**Figure 8:** Showing Linearity curve for ***Ornidazole***

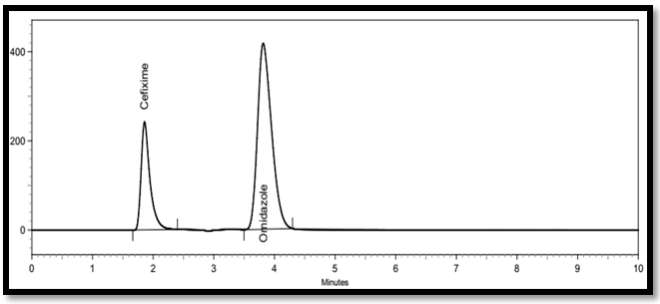


**Figure 9:** Showing Linearity study chromatogram of level-1 (40%)

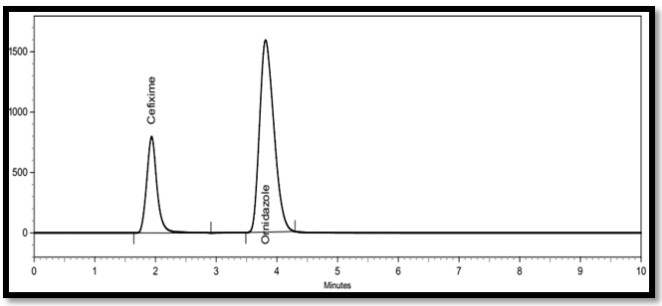


**Figure 10:** Showing Linearity study chromatogram of level-7 (160%)

### 3. Precision

The Data obtain from precision experiments are given in Table 1 for intraday and interday precision study intended for both Cefixime and Ornidazole. The RSD values for intraday precision study and interday precision study was < 2.0 % for Cefixime and Ornidazole. Which confirm that the method was precise?

| Set | Cefixime (%Assay) | | Ornidazole (%Assay) | |
|---|---|---|---|---|
| Intraday (n = 6) | Interday (n = 6) | Intraday (n = 6) | Intraday (n = 6) | Intraday (n = 6) |
| 1 | 101.4 | 100.7 | 99.9 | 98.8 |
| 2 | 101.5 | 101.6 | 100.0 | 99.2 |
| 3 | 99.0 | 101.6 | 99.1 | 99.7 |
| 4 | 100.4 | 100.8 | 100.0 | 99.6 |
| 5 | 100.7 | 99.4 | 101.3 | 99.5 |
| 6 | 99.7 | 100.1 | 100.8 | 100.0 |
| Mean | 100.4 | 100.7 | 100.2 | 99.5 |
| Standard deviation | 0.96 | 0.86 | 0.77 | 0.43 |
| % RSD | 0.96 | 0.85 | 0.77 | 0.43 |

**Table 1:** Showing Results Data of Precision Study

### 4. Accuracy

The Recovery of Cefixime and Ornidazole were determined at three different concentration levels. The mean recovery for Cefixime was 98.42-99.59 % and 98.20-99.59 % for Ornidazole (Table 2). The result indicating that the method was accurate.

| | Level (%) | Amount Added Concentration[a] (mg/ml) | Amount Found Concentration[a] (mg/ml) | % Recovery | % RSD |
|---|---|---|---|---|---|
| Cefixime | 50 | 0.03995 | 0.03932 | 98.42 | 0.68 |
| | 100 | 0.08027 | 0.07994 | 99.59 | 0.06 |
| | 150 | 0.12001 | 0.11903 | 99.18 | 0.25 |
| Ornidazole | 50 | 0.10015 | 0.09913 | 98.99 | 0.73 |
| | 100 | 0.20092 | 0.20009 | 99.59 | 0.43 |
| | 150 | 0.30052 | 0.29510 | 98.20 | 0.34 |

**Table 2:** Showing Results of Accuracy Study

### 5. Robustness

The robustness of the results of research, the development of the content is to build in table 3 and Table 4. The results show that the variance in all conditions, the measured values of test preparation solution is not affected, and it is on the basis of this reality. The system suitability parameters also found a satisfactory; therefore, analysis method will come to the same concluded as robust.

| Robust Conditions | % Assay | System suitabi | Ity parameters |
|---|---|---|---|
| | | Theoretical plates | Asymmetry |
| Flow 0.5 ml/min | 99.7 | 1950 | 1.55 |
| Flow 0.7 ml/min | 99.4 | 1998 | 1.37 |
| Water:ACN:MeoH (48:26:26,v/v) | 99.3 | 2015 | 1.49 |
| W ater: ACN :MeoH (52:24:24,v/v) | 100.8 | 1960 | 1.37 |
| Column change | 100.8 | 1940 | 1.39 |

**Table 3:** Showing Robustness Study

| Robust Conditions | % Assay | System suitabi | Ity Parameters |
|---|---|---|---|
| | | Theoretical plates | Asymmetry |
| Flow 0.5 ml/min | 98.3 | 4520 | 1.55 |
| Flow 0.7 ml/min | 99.2 | 4568 | 1.49 |
| Water:ACN:MeoH (48:26:26,v/v) | 99.2 | 4589 | 1.66 |
| Water:ACN:MeoH (52:24:24,v/v) | 98.8 | 4734 | 1.48 |
| Column change | 99.4 | 4687 | 1.53 |

**Table 4:** Showing Robustness Study

## 1.7 CONCLUSION

A new sensitive; RP-TNC HPLC analytical method development and validation, while at the same time estimates, the CEF and the order of the combined dosage forms. The HPLC method for quantitation of Continuing Education Fund (CEF) is enabled and the orders of oral dose forms have very good accuracy and precision, both in the laboratory to prepare samples or in drug dosage forms.

On the way to get a good recovery in all cases, as well as reliable protocol and will report that the proposed methodology can be applied effectively to determine if the CEF and the order in tablet dosage form with satisfactory accuracy. This method is considered to be a simple, reliable, and cost-effective and provide satisfactory accuracy and precision of the lower limits of detection and quantification of more sensitive. Therefore, we believe that the future of the analysis methods, use this method to estimate the CEF and the total dosage form with no amendment.

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8. P. V. Rege. And Ramesh Mapari : ***Simultaneous Quantification of Ofloxacin And Ornidazole*** From Combined Pharmaceutical Drug Formulation By HPLC.



**Corresponding Author**

**Suman Sharma***

Research Scholar, Department of Chemistry, OPJS University, Rajasthan

**E-Mail –** suman.msc85@gmail.com