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Validated LC Method for Simultaneous analysis of Cefixime and Ornidazole in commercial tablets

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Abstract: A simple, precise, and accurate HPLC method has been developed and validated for assay of combine dosage form of Cefixime and Ornidazole in commercial tablets. Reversed-phase liquid 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. Detection was performed at 304nm. The method was validated for specificity, linearity, precision, accuracy, robustness, and by stress testing of the drug(forced degradation). Response was a linear function of drug concentration in the range *0.08-0.32 mg/ml* (*r*= 0.9992). Intraday and interday system and method precision were determined. Accuracy was between 98.20 and 99.59%. The method was found to be robust, and was suitable for assay of Cefixime and Ornidazole in a tablet formulation.
Keywords: Cefixime and Ornidazole, Stability Indicating Assay, Method Validation.

1. INTRODUCTION OF DRUGS

1.1 Introduction to Cefixime

Cefixime is an oral third generation cephalosporin antibiotic. It is used to treat gonorrhea, tonsilitis, and pharyngitis.

1.1.1 Description

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 [Figure 1]. Its molecular formula is $C_{16}H_{15}N_5O_7S_2$ having molecular weight 453.45 gm/mole. Cefixime is an oral third generation cephalosporin antibiotic. It is used to treat gonorrhea[1], tonsilitis[2], and pharyngitis[2].

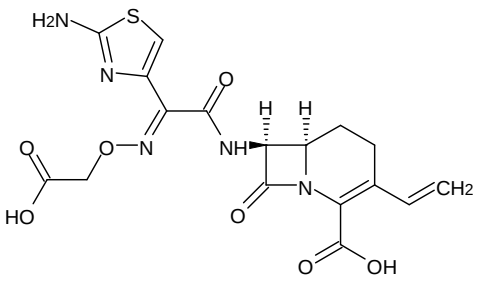

Fig. 1: Chemical Structure of Cefixime

1.2 Introduction to Ornidazole

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[3].

1.2.1 Description

Ornidazole is chemically 1-chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol [Figure 2]. It's molecular formula is $C_7H_{10}ClN_3O_3$ having molecular weight 219.63 gm/mole. 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.

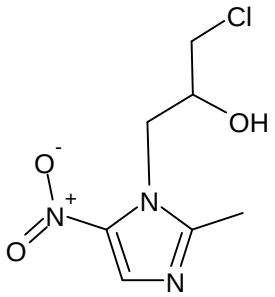

Fig. 2: Chemical Structure of Ornidazole

Various publications are available regarding 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. Voltammetric determination, fluorescence spectrophotometry, thin-layer chromatography, first-derivative and spectrophotometric and HPLC methods are reported. Only three methods are reported for the simultaneous determination of Cefixime and Ornidazole. It's methods are UV spectrophotometric determination, which is able to determine cefixime and ornidazole in combined dosage form[4-24]. So far as our knowledge is concern, no HPLC analytical method for the determination of cefixime and ornidazole in combine dosage forms has been published. The previous published methods are not directly applicable for this issue and need more investigation for method development and validation.

Consequently, the focus in the present study was to develop a validated simple, accurate and precise HPLC method for the combination.

The aim and scope of the proposed work are as under:

- To develop suitable HPLC method for simultaneous determination cefixime and ornidazole in tablet formulation.
- Perform the validation for the developed method.

3. EXPERIMENTAL

3.1 Materials

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 Pvt. Ltd., Mumbai (India).

3.2 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).

4.3 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 Pvt. Ltd. Bangalore, India) and degassed in an ultrasonic bath (Spincotech Pvt. Ltd., Mumbai).

4.4 Diluent preparation : Methanol used as diluents.

4.5 Standard preparation

Cefixime standard stock solution containing 2000µg/ml was prepared in a 100 ml volumetric flask by dissolving 200.00 mg of cefixime 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 80µg/ml). 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).

4.6 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. About 50 ml of diluent was added and sonicated for a minimum 30 minute with intermittent shaking. Then 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.

4.7 Chromatographic Conditions

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. Detection was performed at 304nm.

5. RESULTS AND DISCUSSION

5.1 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 column 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 selection of water, acetonitrile, methanol based on chemical structure of both the drugs.These solvent composition was 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 : 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.

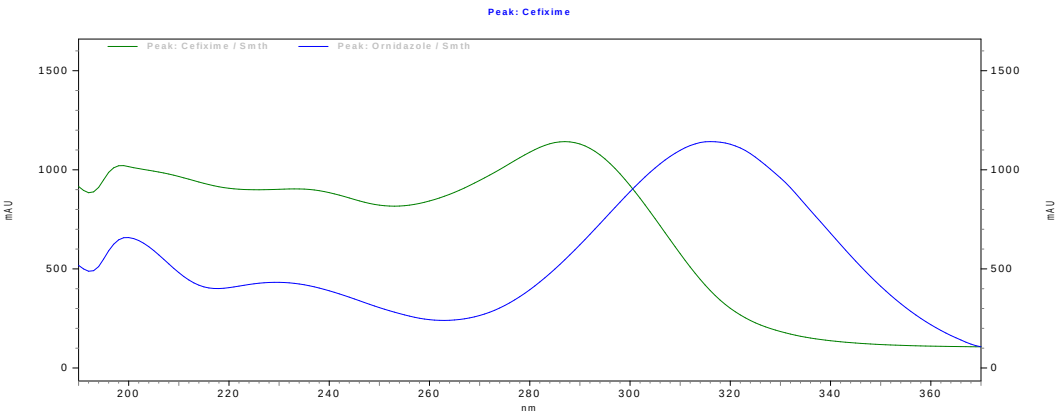

Figure 3: Wavelength scan overlay of standard preparation

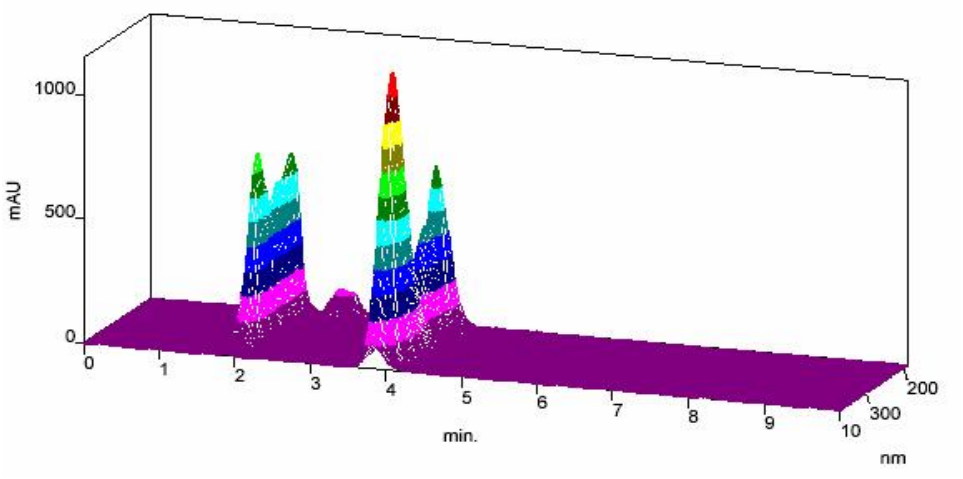

Figure 4: PDA scan of standard preparation

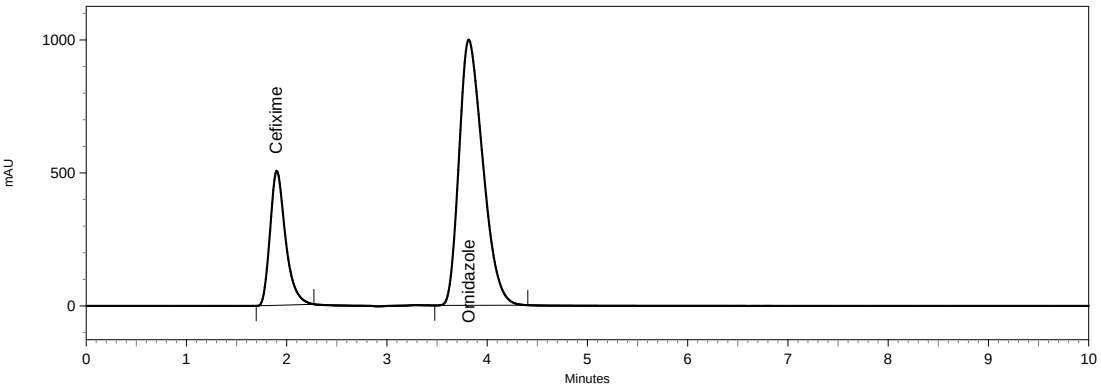

Figure 5: Chromatogram of standard preparation

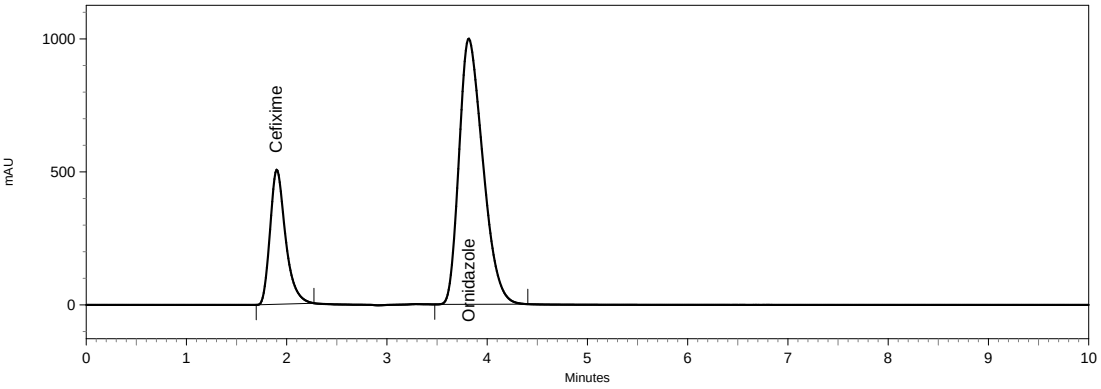

Figure 6: Chromatogram of test preparation

5. Method Validation

5.3.1 Specificity

In an assay, demonstration of specificity requires that it can be shown that the procedure is unaffected by the presence of impurities or excipients. In practice, this can be done by spiking the drug substance or product with appropriate levels of impurities or excipients and demonstrating that the assay results is unaffected by the presence of these extraneous materials.

There should be no interference of the diluents , placebo at retantion time of drug substances.

5.3.2 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. Chromatogram obtain during linearity study were shown in Figure 9-15.

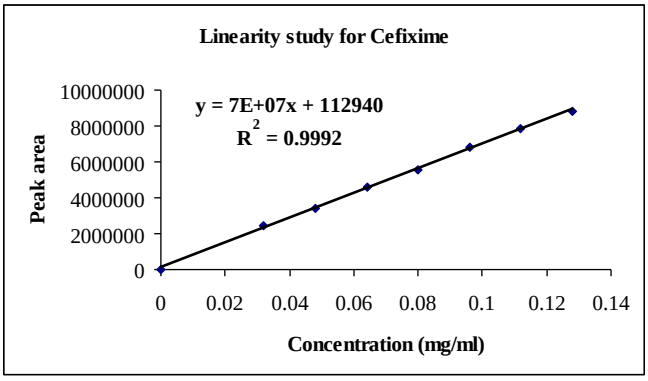

Figure 7: Linearity curve for Cefixime

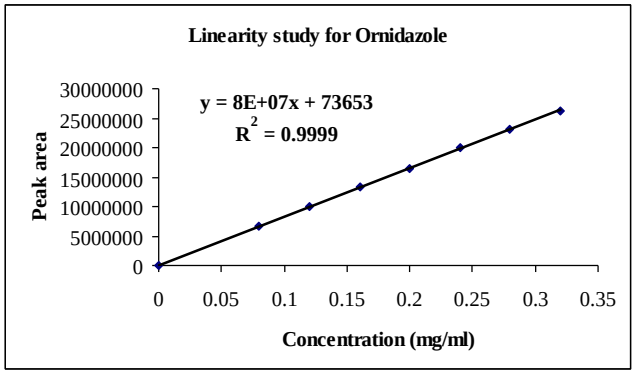

Figure 8: Linearity curve for Ornidazole

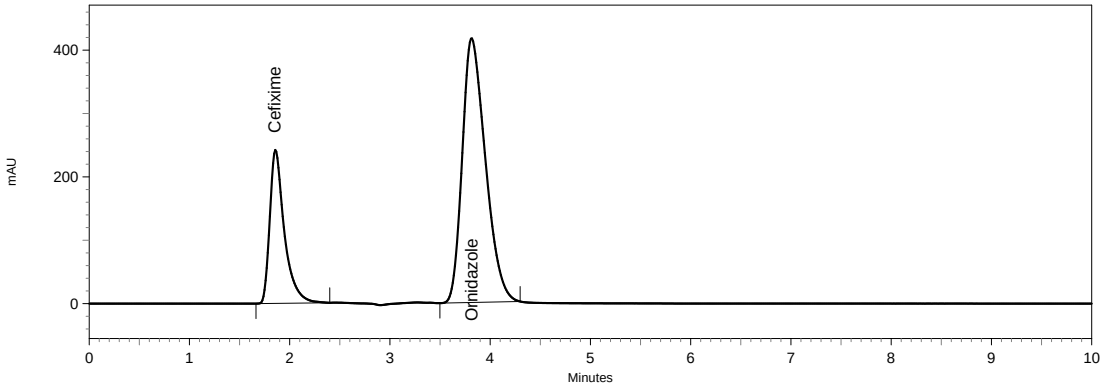

Figure 9: Linearity study chromatogram of level-1 (40%)

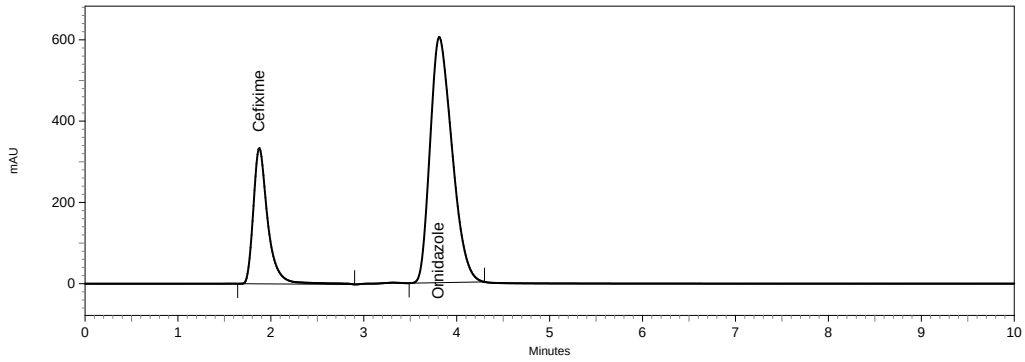

Figure 10: Linearity study chromatogram of level-2 (60%)

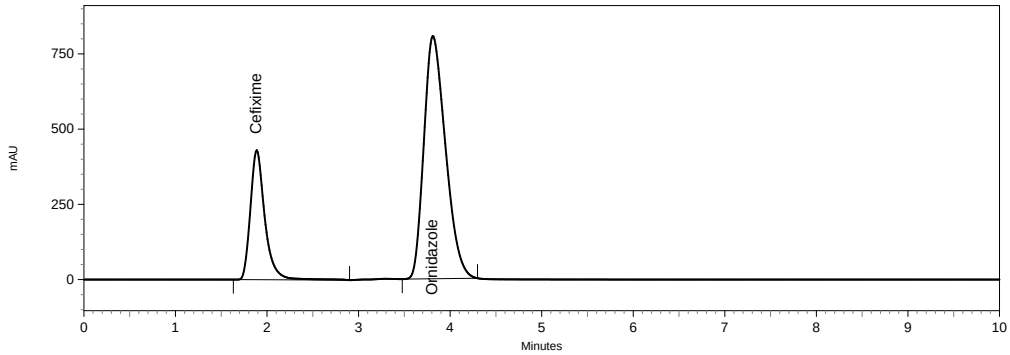

Figure 11: Linearity study chromatogram of level-3 (80%)

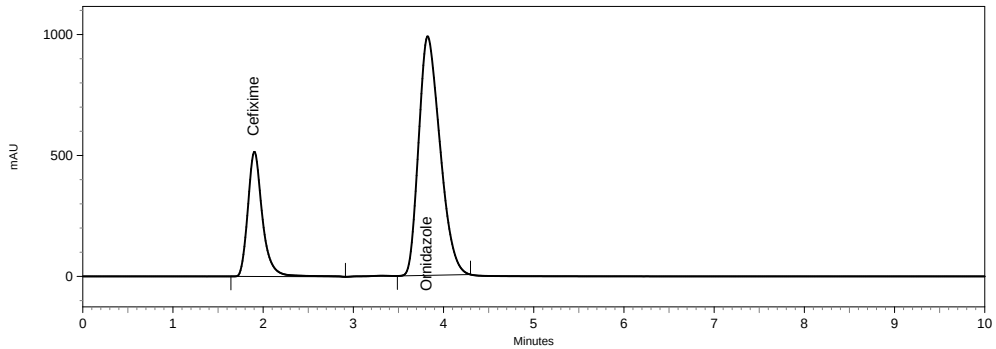

Figure 12: Linearity study chromatogram of level-4 (100%)

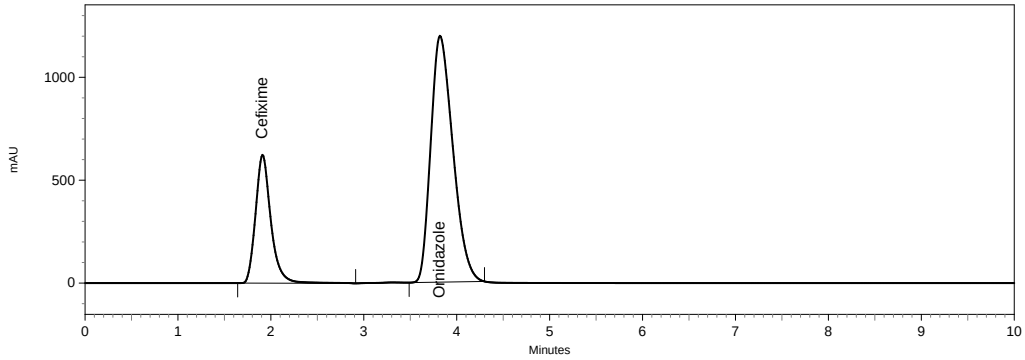

Figure 13: Linearity study chromatogram of level-5 (120%)

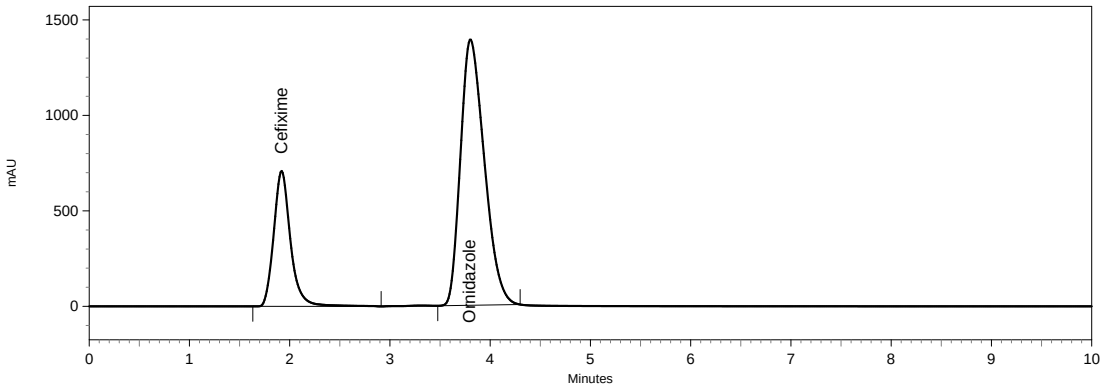

Figure 14: Linearity study chromatogram of level-6 (140%)

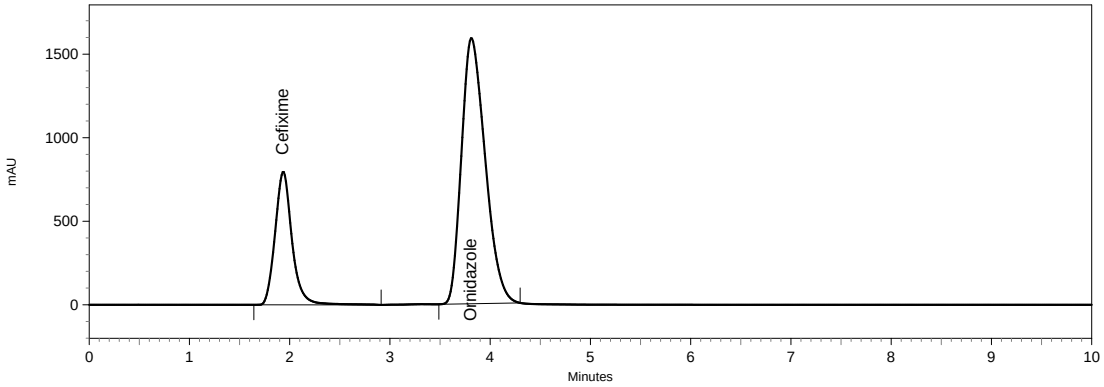

Figure 15: Linearity study chromatogram of level-7 (160%)

5.3.3 Precision

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

Table 1: Results of precision study

Set	Cefixime (%Assay)		Ornidazole (%Assay)	
	Intraday (n = 6)	**Interday (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

5.3.5 Accuracy

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. Chromatogram obtain during accuracy study were shown in Figure 16-18.

Table 2: Results of accuracy study

	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

[a] Each value corresponds to the mean of three determinations

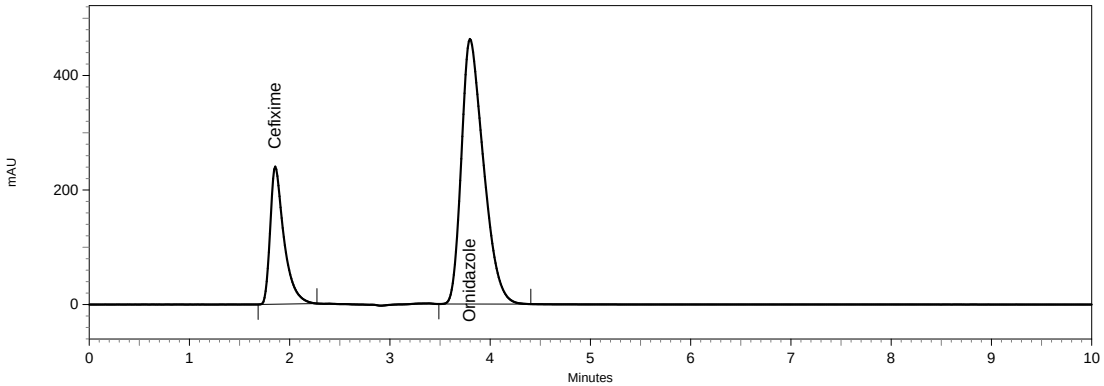

Figure 16: Accuracy study chromatogram of level-1 (50%)

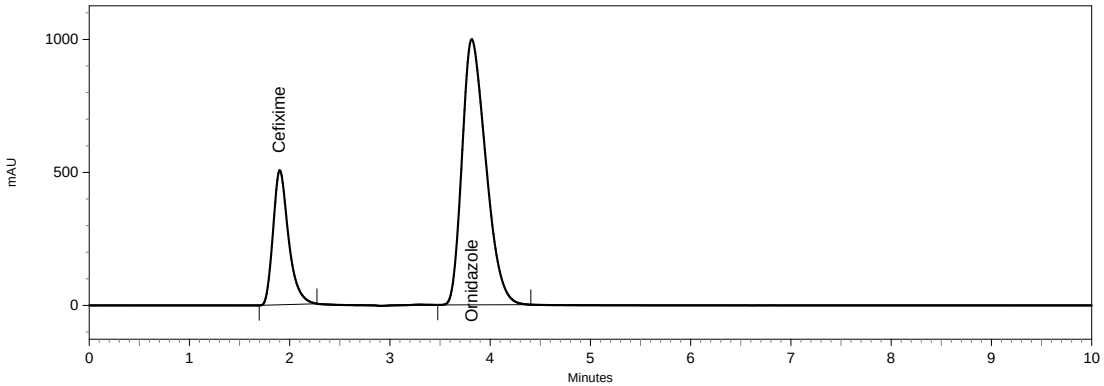

Figure 17: Accuracy study chromatogram of level-2 (100%)

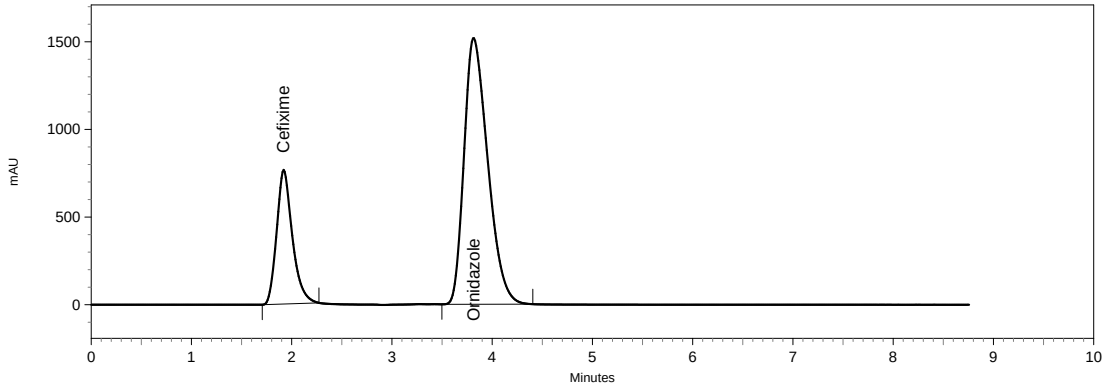

Figure 18: Accuracy study chromatogram of level-3 (150%)

5.3.6 Solution stability study

Table 3 shows the results obtain in the solution stability study at different time intervals for test preparation. It was concluded that the test preparation solution was found stable up to 48 h at 2 - 8˚ C and ambient temperature with the consideration of < 2.0 % in %assay value difference against interval value.

5.3.7 Robustness*:*

The result of robustness study of the developed assay method was established in Table 4 and Table 5. The result shown that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory, hence the analytical method would be concluded as robust. Chromatogram obtain during robustness study were shown in Figure 19-23.

Table 3: Evaluation data of solution stability study

Intervals	%Assay for test solution stored at 2-8˚ C		% Assay for test solution stored at ambient temperature	
	Cefixime	Ornidazole	Cefixime	Ornidazole
Initial	100.98	99.24	100.98	99.24
12 h	100.82	98.38	100.68	98.72
24 h	101.24	98.58	100.59	98.30
36 h	100.49	98.20	100.27	98.05
48 h	100.68	98.21	100.42	98.03

Table 4: Evaluation data of robustness study of Cefixime

Robust conditions	% Assay	System suitability 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
Water:ACN:MeoH (52:24:24,v/v)	100.8	1960	1.37
Column change	100.8	1940	1.39

Table 5: Evaluation data of robustness study for Ornidazole

Robust conditions	% Assay	System suitability 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

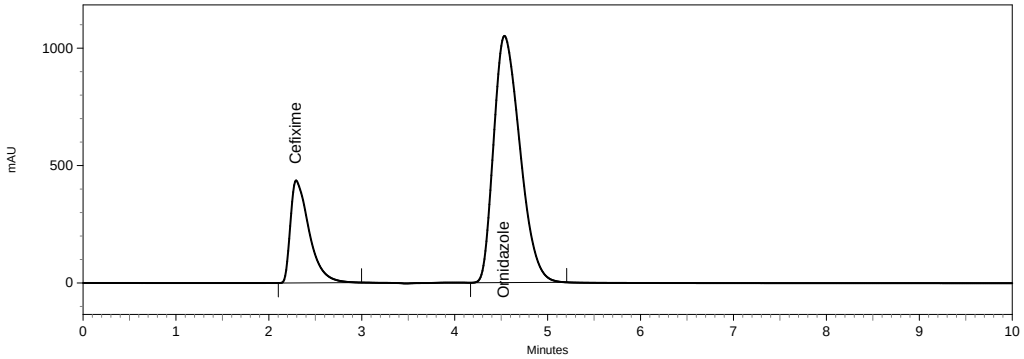

Figure 19: Standard chromatogram (0.5 ml/min flow rate)

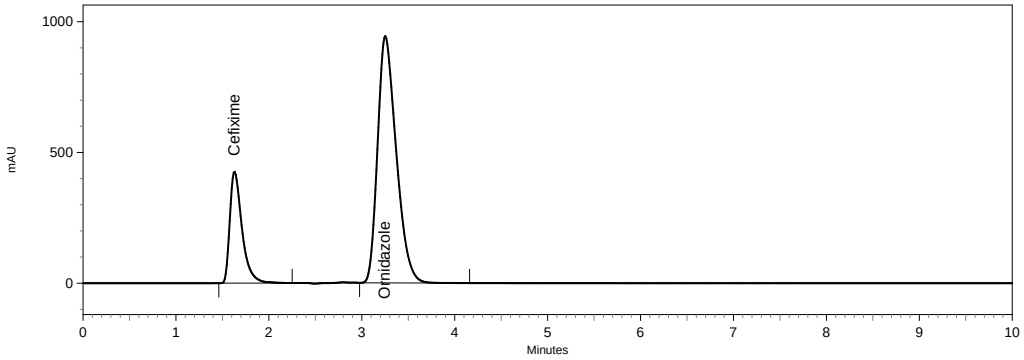

Figure 20: Standard chromatogram (0.7 ml/min flow rate)

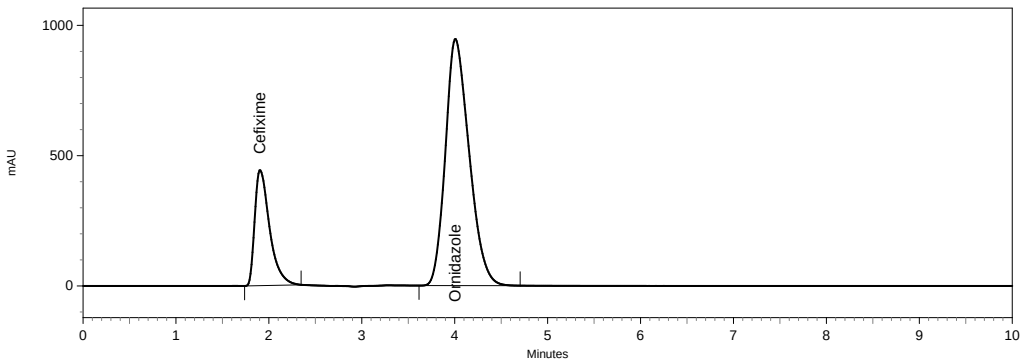

Figure 21: Standard chromatogram (Water: ACN: MeoH, 48:26:26,v/v)

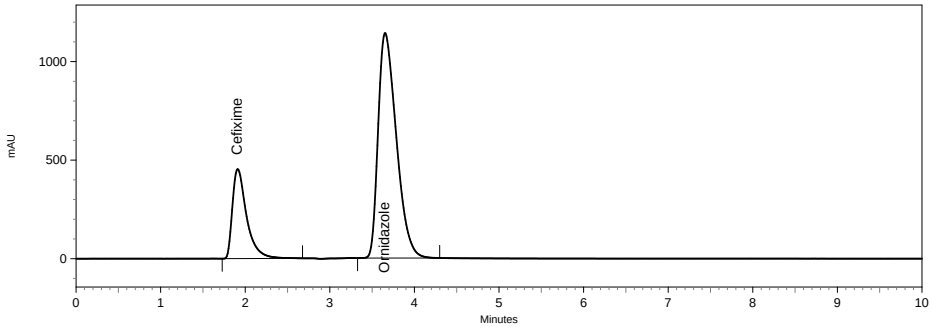

Figure 22: Standard chromatogram (Water: ACN: MeoH, 52:24:24,v/v)

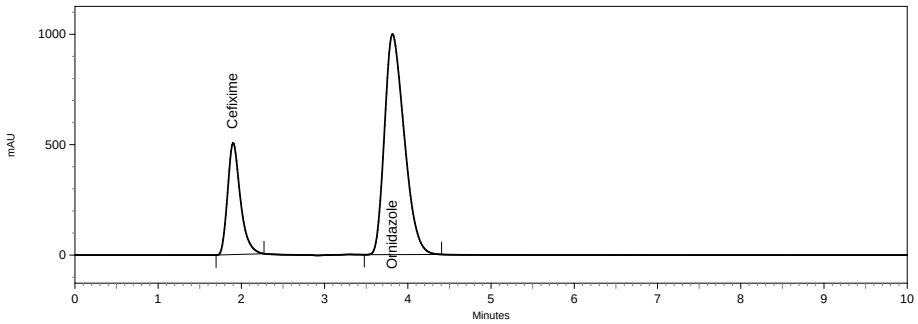

Figure 23: Standard chromatogram (Column change)

5.3.8 System suitability:

A system suitability test of the chromato graphic system was performed before each validation run. Five replicate injections of standard preparation were injected and asymmetry, theoretical plate, resolution and % RSD of peak area were determined for same. Acceptance criteria for system suitability, Asymmetry not more than 2.0, theoretical plate not less then 1800 for Cefixime and 4000 for Ornidazole and % RSD of peak area not more then 2.0, were full fill during all validation parameter.

Comparison for Precision and Intermediate Precision Study for Analytical Method Validation for Cefixime and Ornidazole Tablets

For Cefixime

	Set	%Assay
Precision study	1	101.4
	2	101.5
	3	99.0
	4	100.4
	5	100.7
	6	99.7
Intermediate precision study	1	100.7
	2	101.6
	3	101.6
	4	100.8
	5	99.4
	6	100.1
	Mean	100.6
	Stdev	0.88
	% RSD	0.88

For Ornidazole

	Set	% Assay
Precision study	1	99.9
	2	100.0
	3	99.1
	4	100.0
	5	101.3
	6	100.8
Intermediate precision study	1	98.8
	2	99.2
	3	99.7
	4	99.6
	5	99.5
	6	100.0
	Mean	99.8
	Stdev	0.70
	% RSD	0.70

6. Conclusion

This LC method for assay and determination of content uniformity of Cefixime and Ornidazole in a tablet formulation was successfully developed and validated for its intended purpose. The method was shown to specific, linear, precise, accurate, and robust. Because the method separates Cefixime and Ornidazole and all the degradation products formed under variety of stress conditions it can be regarded as stability indicating. There is no method reported for determination of assay and content uniformity of Cefixime and Ornidazole in pharmaceutical dosage forms. This method is recommended to the industry for quality control of drug content in pharmaceutical preparations

7. Acknowledgements

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