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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF CEFIXIME AND ORNIDAZOLE IN BINARY COMBINATION AND MARKETED FORMULATIONS

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ABSTRACT

Background: Cefixime is a tird generation cephalosporin antibiotic and Ornidazole is a imidazole derivation antibacterial agent. Both in fixed dose combination is used in management of GIT disorder specially diarrhoea. HPLC is precise and accurate sophisticated instrumentation technique used routinely in analysis of pharmaceuticals and other chemicals. **Objective**: No HPLC method have been reported for Simultaneous Determination of Cefixime and Ornidazole, hence this study aims to develop and validate a simple high-performance liquid chromatography (HPLC) method for the simultaneous determination of Cefixime and Ornidazole. **Method**: Isocratic elution was carried out in water and ACN (75:25) with 0.3% Triethyamine, Final pH adjusted to 3.2 ± 0.1 with 10 % o-phosphoric acid. The flow rate was kept 1 mL/min for 10 min and detection wavelength at 310 nm. The method has been validated according to International Conference of Harmonization (ICH) Q2 (R1) guidelines with respect to specificity, system suitability, accuracy, precision, and robustness. The developed method was further used for the determination of CFX and OZ in Marketed formulation. **Result**: The method was linear in the concentration range of 01-70 mg/mL for Cefixime and Ornidazole. The limit of detection (LOD) and limit of quantification (LOQ) were 0.37 and 1.23 µg mL for cefixime, respectively, while LOD and LOQ for Ornidazole were 0.41 and 1.37 µg/respectively. The percentage recovery that was found to be in the range of 99-102% with relative standard deviation less than 2% indicating the accuracy and precision of method for

both the drugs. Further, the validated method was found specific to detect presence of both the drugs in extracts, marketed formulations. **Conclusion**: The developed method showed excellent specificity, linearity, accuracy and precision. Thus, it can be further explored to detect curcumin and quercetin in bulk powder mixture as well as other marketed formulations.

KEYWORDS: Cefixime, RP-HPLC, Ornidazole, Validation.

1. INTRODUCTION

Change in Secretion and motility in GIT may cause GIT disturbances. Achlorhydria, Hyperchlorhydria, Constipation and Diarrhoea are some common GI disturbance. Antibacterial agents are prescribed in management of Diarrhoea if it is due to GIT infection.[1] Ornidazole is an antimicrobial agent for the treatment of infections due to *Trichomonas vaginalis*, *Entamoeba histolytica* and *Giardia lamblia* (Giardia intestinalis) and also against certain anaerobic bacteria such as *Bacteroides* and *Clostridium spp.*, *Fusobacterium spp*., and *anaerobic cocci*.[2,3] Ornidazole inhibits the growth of protozoa by interacting with the DNA of the micro-organism and inhibiting the protein synthesis, thereby leading to death of the micro-organism.[4] Ornidazole (Fig 1) is chemically described α-(Chloromethyl)-2-methyl-5-nitroimidazole-1-ethanol. So Ornidazole is 5-nitroimidazole derivative in which the hydrogens at positions 1 and 2 are replaced by 3-chloro-2-hydroxypropyl and methyl groups. It has PKa, 2.4 ± 0.1.[5]

Cefixime is a semi synthetic, third-generation cephalosporin for oral administration that has been shown to be highly active against a broad range of gram negative some gram positive aerobic bacteria.[6,7] The bactericidal action of Cefixime is due to the inhibition of cell wall synthesis.[8] Cefixime is used to treated certain infection caused by bacteria like Bronchitis, Gonorrhea, infections of the ears, throat, lungs, urinary tract, Typhoid fever. Cefixime is chemically designated as (6R, 7R) 7-{[2-(2-Amino-1,3-thizol-4yl)-2 (carboxymethoxyimino) acetyl]amino}-3-ethenyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid.[9]

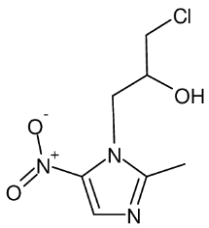


Fig. 1: Ornidazole.

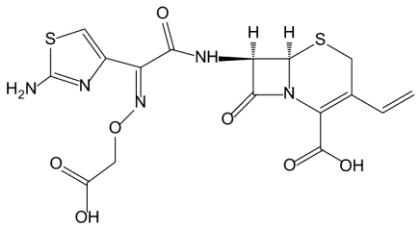


Fig. 2: Cefixime.

A 'regulatory analytical procedure' is used to evaluate a defined characteristic of the drug substance or drug product. An 'alternative analytical procedure' is proposed by the applicant for use other than regulatory analytical procedure. The modern methods of choice for quantitative analysis are High performance liquid chromatography (HPLC), GLC and HPTLC, which are highly sophisticated. HPLC is the fastest growing analytical technique for the analysis of drugs.[10] Its simplicity, high specificity, and wide range of sensitivity make it ideal for the analysis of many drugs in both dosage forms and biological fluids. OZ and CFX are available as single component or in combined dosage forms.[11] Many alternative analytical methods either in pharmaceutical products or in biological samples are reported in the literature for the determination of OZ alone[12,16], CFX alone[17,19] OZ with other drugs[20,22], CFX with other drugs[23,24] and OZ and CFX in combination.[25,26] So, it was aim of present study to develop and validate an accurate, precise, robust, simple and rapid RP-HPLC method for Simultaneous Determination of Cefixime and Ornidazole in binary combination and Marketed Formulations.

2. MATERIAL AND METHODS

2.1: Material, reagents and pharmaceutical products

Pharmaceutical grade of Ornidazole (OZ), Cefixime (CFX) and reference standards were kindly supplied as a gift sample by Torrent Research Center, Ahmedabad, India. Acetonitrile, Water used was of HPLC grade. (Rankem). Triethylamine and *o*-phosphoric acid (AR grade, S.D. Fine Chemicals Ltd., Mumbai). Combined tablets of Cefixime and Ornidazole were procured from local market.

2.2 Instrumentation

Merck - Hitachi isocratic High Performance Liquid Chromatography system comprising of, Hitachi pump L- 7110, Rheodyne universal injector 77251 with injection volume 20 µl, Hitachi L - 7420 UV - Visible Detector, Merck - Hitachi HSM software and LiChrospher® 100 rp-180, c18, column having 250 mm length, 4.0 mm internal diameter and 5 µm particle

size. Shimadzu model 1601 double beam UV - Visible Spectrophotometer with a pair of 10 mm matched quartz cells. Shimadzu – libror 220 balance. Ultrasonic bath (Frontline fs 4 ultrasonic cleaner). Digital pH meter (Analab). Corning Volumetric flasks (10, 25, 50, 100, 250 ml).

2.3 Spectral Analysis: [wave length selection]

Standard solution of drug sample OZ and CFX (1 mg mL^{-1}) in water and their mixture (100 mg mL^{-1} each) were prepared in mobile phase, and it was scanned for the determination of absorption (UV -1601, Double beam, Shimadzu, Japan) in the range of 200-400 nm against mobile phase as blank. In addition to that the solution of their physical mixture was also scanned in the same range. OZ and CFX showed reasonably good absorbance at 310 nm. So the wavelength selected for the quantification of OZ and CFX was 310 nm.

2.4 Analytical Method Development: [Chromatographic conditions and Instrumentation]

The analytical method was developed by using an HPLC system (Merck - Hitachi isocratic HPLC system) with a Hitachi L - 7420 UV - Visible Detector and a Rheodyne universal injector (77251) equipped with an L-7110 Hitachi pump. Statistical acquisition, recording, and chromatographic integration were achieved using Merck - Hitachi HSM software. Analysis and separation have been done on the LiChrospher® 100 rp-180, c18, column having 250 mm length, 4.0 mm internal diameter and 5 µm particle size. Isocratic elution was carried out using water and ACN as the mobile phase. The mobile phase consisted of Water: Acetonitrile: Triethylamine (75:25:0.3, v/v/v), final pH adjusted to 3.20 ± 0.02 with 10 % v/v *o*-phosphoric acid in a isocratic mode and was pumped at a flow rate of 1.0 ml/min. The mobile phase was filtered through nylon 0.45 µm, 47 mm membrane filter and was degassed before use. The elution was monitored at 310 nm. The injection volume was 20 µl.

2.5 Preparation of solutions

Standard OZ stock solution (1 mg/ml) and Standard CFX stock solution (1 mg/ml) were prepared by transferring accurately weighed OZ (25 mg) and CFX (25 mg) to two separate 25 ml volumetric flasks, dissolved and diluted up to the mark with water.

Mixed standard stock solution of OZ and CFX (100 µg/ml) was prepared by transferring 10 ml aliquots each from stock solutions of OZ and CFX in to a 100 ml volumetric flask and

volume was made up with mobile phase up to mark to get 100 µg/ml mixed standard stock solution.

Bulk powder solution was prepared by transferring accurately weighed OZ (25 mg) and CFX (10 mg) was transferred to a 100 ml volumetric flask and dissolved in and diluted to mark with water. The solution (2.0 ml) was transferred to a 10 ml volumetric flask and diluted to the mark with mobile phase to obtain final solution with OZ (50 µg/ml) and CFX (20 µg/ml).

Sample solution was prepared as follow: Twenty tablets were weighed, their average weight was determined, and crushed in mortar. An amount of powdered mass equivalent to 10 mg of CFX or 25 mg of OZ was weighed and transferred in 100 ml volumetric flask and mixed with 50 ml of water. To ensure complete extraction of drugs it was sonicated for 30 min. The solution was filtered through Whatman filter paper No. 41 and the residue was washed thoroughly with 10 ml water. The filtrate and washings were combined in a 100 ml volumetric flask and diluted to the mark with water. Two ml aliquot from above solution was transferred in 10 ml volumetric flask and volume was adjusted with mobile phase up to mark to achieve sample solution with CFX (20 µg/mL) and OZ (50 µg/mL).

2.6 Method development and optimization: [Selection of Mobile phase and column]

various mobile phases and elution methods were initially tried in order to have both eluents on the same chromatogram. The mobile phase composition used in the optimized method have been decided based on sensitivity and selectivity as well as suitable chromatographic parameters of the developed peaks in terms of peak shape, peak purity, peak sharpness, tailing factor, and resolution between the two peaks. The mobile phase was used as a solvent for all samples to confirm least noise and to eradicate any undesirable solvent peaks. Various trials were carried out in order to find better chromatographic conditions. The concentration of CFX and OZ for developing the analytical method was 10 mg mL^{-1}. Knowledge of the molecule suggested that Reverse Phase Liquid Chromatography (RPLC) would be suitable for the simultaneous analysis of OZ and CFX. Resolution is the most important criteria for the method, and is imperative to achieve good resolution among the both compounds. As per the value of Ka and solubility of both the compounds, various compositions of mobile phase with different pH ranges (2.75 to 7.0) were tried. Best resolution was obtained with mobile phase consisting of water, acetonitrile and triethylamine in the ratio of (75:25:0.3, v/v/v), apparent pH adjusted to 3.2 ± 0.1 with 10 % *o*-phosphoric acid.

2.7 Method Validation

The optimized method for the simultaneous estimation of OZ and CFX has been validated as per ICH Q2 (R1) guidelines.[27] The developed method was validated in 8 of linearity, accuracy, limit of detection, limit of quantification, intra-day and inter-day precision and repeatability of measurement, robustness, specificity as well as repeatability of sample application.

2.7.1 System suitability

The system suitability was assured by determining the peak retention time, peak area, theoretical plates, and tailing or asymmetry factor for OZ and CFX.

Table 1: system suitability parameters with acceptance criteria.

Sr. No	Parameter name	Acceptance criteria
1	Number of theoretical plates or Efficiency (N)	> 2000
2	Capacity factor (K)	< 1
3	Separation or Relative retention (α)	> 1
4	Resolution (Rs)	> 1.5
5	Tailing factor or Asymmetry(T)	< 2
6	Relative Standard Deviation (RSD)	< 2

The standard concentration of 20µg/ml CFX and 50µg/ml OZ was used to prepare the samples. The sample preparation was accomplished in accordance with the method described above in Section 2.5. Six replicate samples were assayed to determine the system's suitability.

2.7.2 Linearity and range

Appropriate aliquots from standard stock solution of mixed drugs were suitably diluted with mobile phase in such a way to get concentrations in a range of 1- 100 µg/ml for both drugs. These Solutions (n=5) were injected in to the universal injector 77251 (Rheodyne) with injection volume 20 µl. Evaluation of two drugs was performed with UV/Visible detector at 310 nm. Peak areas were recorded for all the peaks. The plots of peak area verse the respective concentration of OZ and CFX was drawn.

2.7.3 Determination of LOD and LOQ

The limit of detection and the limit of quantification of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines.

$$LOD = 3.3 \times \sigma/S \text{ and } LOQ = 10 \times \sigma/S$$

Where, σ = standard deviation of the response S = slope of the calibration curve

2.7.4 Accuracy study

This was carried out to check the recovery of the drugs at three different levels in the formulations i.e. multilevel recovery study. The pre analyzed samples were spiked with extra 50, 100, 150 % of the standard CFX and OZ, respectively and the mixtures were analyzed by proposed method. The experiment was repeated for five times (n=5).

2.7.5 Precision studies

Method precision (Repeatability)

Repeatability of sample was carried out. Measurements of peak area, retention time, tailing factor and asymmetry were carried out by using six replicates of same concentration (20 µg/ml of OZ and CFX). Percentage Relative Standard Deviation (RSD) or Coefficient of Variation (CV) should be less than 2%.

Intermediate precision (Reproducibility)

It expresses within laboratory variations as on different days analysis or equipment within the laboratory. The intra- and inter-day variation for the determination of OZ and CFX were carried out at four different concentration levels 10, 20, 30 and 40µg/ml.

2.7.6 Robustness

Robustness of the method was assessed by examining changes in different experimental conditions. Six sample solutions were prepared and analyzed using the established conditions and by varying some of the chromatographic conditions. Changes in mobile phase pH (±0.2 pH units), solvent composition in mobile phase (±2 %), wavelength (±2 nm), and Flowrate (±0.1) were made and data obtained. Robustness of the method was done at four different Cconcentration 10µg/ml OZ and 10µg/ml CFX strength used in Robustness study. The mean peak area was determined for OZ and CFX and the % CV was calculated (n=3).

2.7.7 Method specificity

Specificity of the developed method was evaluated by preparing a solution of the reference standards of the two APIs in the presence of excipients. Five injections of this solution were carried out to observe any interfering peaks.

2.7.8 Solution stability

Sample solutions and were kept at 25^0C and 2-8^0C for 24 h and 72 days, respectively. Assay of initial time period was compared with these two time points. The falls in the assay values

were evaluated. The difference between assays should not be more than 2 % for formulation, and 0.5 % for Active Pharmaceutical Ingredients.

Method applicability

The applicability of the method has been confirmed where the optimized conditions were applied to quantify CFX and OZ in bulk powder mixture and marketed formulations.

3. RESULTS AND DISCUSSION

3.1 Selection of UV Wave length

OZ has an absorption maximum around 319 nm, and CFX has absorption maxima at 288 nm. OZ and CFX showed reasonably good absorbance at 310 nm. So the wavelength selected for the quantification of OZ and CFX was 310 nm.

3.2 Method Development and Optimization

As per the value of Ka and solubility of both the compounds, various compositions of mobile phase with different pH ranges (2.75 to 7.0) were tried and best resolution was obtained with mobile phase consisting of water, acetonitrile and triethylamine in the proportion of 75:25:0.3 (v/v/v) with final pH adjusted 3.2 ± 0.02 with 10% v/v o-phosphoric acid. A satisfactory separation and peak symmetry for CFX and OZ were obtained with above mentioned mobile phase Quantification was achieved with UV detection at 310 nm based on peak area. The results of the validation and system suitability results are given in Table 1. A representative chromatogram is shown in Figure 3. Parameters of chromatogram are shown in Table 2. Better resolution of the peaks with clear base line separation was found.

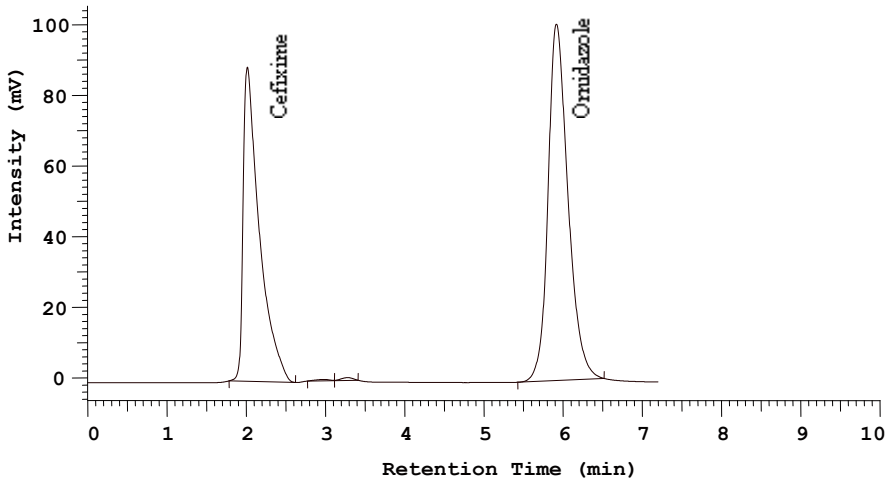


Figure 3: Chromatogram of OZ and CFX with corresponding retention time at 310 nm.

Retention time for OZ and CFX was 5.92 min and 2.02 min, respectively. Asymmetric factor for OZ and CFX was 1.43 and 1.70, respectively. The values of tailing factor for OZ and CFX was 1.27 and 1.32, respectively. Hence both the drugs are better resolved and separated with above mentioned mobile phase. Quantification was achieved with UV detection at 310 nm based on peak area.

3.3 VALIDATION

3.3.1 System Suitability

The obtained results of hexaplicate injections for both the drugs exhibited that the limits tested were within the acceptable range. OZ and CFX were repeatedly retained and well separated at 5.92 and 2.02 minutes, stating very good resolution between both peaks. System suitability parameters of chromatogram for OZ and CFX are given in table 2.

Table 2: System suitability parameters of chromatogram for OZ and CFX.

Parameters	OZ	CFX
Retention time (min)	5.92	2.02
Tailing factor	1.27	1.32
Asymmetry	1.43	1.70
Theoretical plates	3106.51	1868.22

3.3.2 Linearity and range

Linear correlation was obtained between peak areas and concentrations of OZ and CFX in concentration range of 1-70 µg/ml. The linearity of the calibration graphs was validated by the high value of correlation coefficients of the regression equation (r^2 = 0.9991 for OZ and 0.9988 for CFX).

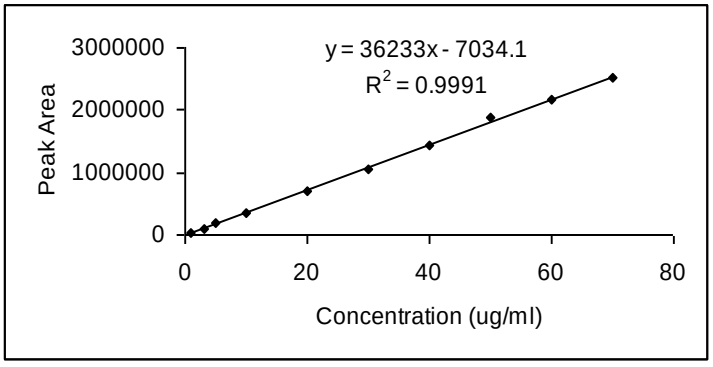


Figure 4: Calibration curve of Ornidazole.

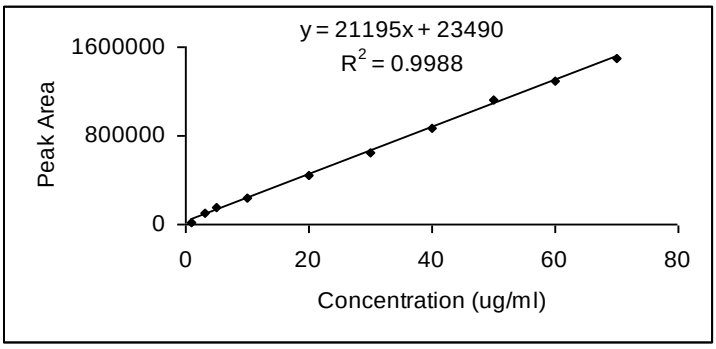


Figure 5: Calibration curve of Cefixime.

Characteristic parameters for regression equation and correlation are given in Table 3.

Table 3: Optical and regression characteristics for analysis of OZ and CFX by RP-HPLC method.

Parameters	OZ	CFX
Concentration range (µg/ml)	1-70	1-70
Limit of Detection (LOD) (µg/ml)	0.37	0.41
Limit of Quantification (LOQ) (µg/ml)	1.23	1.37
Regression equation (y*= a + bc) Slope (b) Intercept (a)	 36233 - 7034.1	 21195 +23490
Regression coefficient (r^2)	0.9991	0.9988
y*= a + bc, where c is the concentration		

3.3.3 LOD and LOQ

The limit of detection and the limit of quantification of the drugs were calculated as in the text. LOD for OZ and CFX were found to be 0.37µg/ml and 0.41 µg/ml, respectively. LOQ for OZ and CFX were found to be 1.23 µg/ml and 1.37 µg/ml respectively (Table 3).

3.3.4 Accuracy study

It was carried out by recovery study using standard addition method. Known amount of standard CFX and OZ were added in to pre-analyzed sample and subjected them to the proposed HPLC method. The study was done at three different concentration levels, i.e. multilevel recovery study.

The percent recoveries obtained for Ornidazole was between 99.72-101.86 % and for Cefixime was 99.90-100.75 %. The low value of SD 0.26-1.54 for OZ and 0.59-1.75 for CFX indicates that the proposed method is accurate. Results of recovery studies are shown in Table 6.3.

Table 4: Recovery data for the proposed method (n=5).

Drug	Amount taken (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery ± S.D (n=5)
Ornidazole	24	12	36.30	101.86 ± 1.54
	24	24	47.84	99.72 ± 0.26
	24	36	60.13	100.22 ± 0.42
Cefixime	10	5	15.05	100.28± 0.89
	10	10	20.15	100.75 ± 0.59
	10	15	24.97	99.90 ± 1.75

3.3.5 Precision studies

3.3.5.1 Method precision

Relative standard deviation of all the parameters was found to be less than 2 % (0.85-1.82), which indicates that the proposed method is repeatable (Table 5).

Table 5: Method precision data for analysis of OZ and CFX by RP-HPLC method.

OZ and CFX (20 µg/ml)	Retention time (min)		Peak area		Asymmetry		Tailing factor	
	OZ	CFX	OZ	CFX	OZ	CFX	OZ	CFX
1	5.92	2.02	698201	439961	1.43	1.7	1.27	1.32
2	5.98	2.06	697412	437512	1.42	1.74	1.28	1.32
3	5.86	1.96	696864	448791	1.4	1.66	1.31	1.34
4	5.93	2.03	694000	440006	1.45	1.72	1.24	1.33
5	5.96	2.04	704300	432147	1.43	1.73	1.26	1.31
6	5.84	2.02	710245	439961	1.47	1.69	1.27	1.28
Mean	5.92	2.02	700170	439729	1.43	1.71	1.271	1.322
SD	0.055	0.034	5984.06	5379.7	0.024	0.029	0.023	0.021
% CV	0.93	1.67	0.85	1.22	1.69	1.72	1.82	1.57

3.3.5.2 Intermediate precision

It was determined as in the text. The low % CV values of intra-day (0.57-1.19 for OZ and 0.69-0.78 for CFX) and inter-day (0.90-1.23 for OZ and 0.59-1.06 for CFX) precision revealed that the proposed method is precise (Table 6a and 6b).

Table 6a: Intra-day precision data for analysis of CFX and OZ by RP-HPLC method.

Concentration		Intra-day precision			
OZ (µg/ml)	CFX (µg/ml)	OZ		CFX	
		Mean ± SD (n=5)	% CV	Mean ± SD (n=5)	% CV
10	10	342329 ± 2106.41	1.193	237157.8 ± 2164.76	0.615
20	20	698155 ± 3783.05	0.831	439978 ± 2438.86	0.788
30	30	1054842 ± 8614.85	0.927	646408 ± 2802.1	0.726
40	40	1437594 ± 14347.41	0.57	867202 ± 8994.97	0.698

Table 6b: Inter-day precision data for analysis of CFX and OZ by RP-HPLC method.

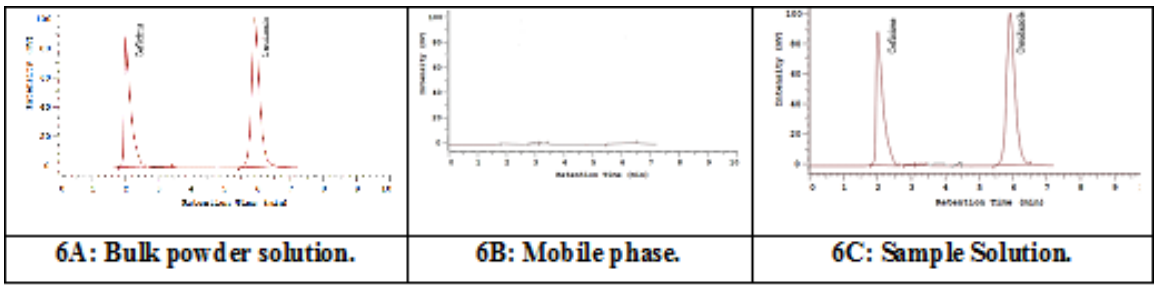
Concentration		Inter-day precision			
OZ (µg/ml)	CFX (µg/ml)	OZ		CFX	
		Mean ± SD (n=5)	% CV	Mean ± S.D (n=5)	% CV
10	10	342877 ± 4180.59	1.235	238717.4 ± 2462.61	1.067
20	20	693496 ± 10837.62	0.950	439683.4 ± 6013.36	0.945
30	30	1049121 ± 13877.67	0.906	644898.6 ± 7266.23	0.596
40	40	1434478 ± 17668.88	1.077	861645 ± 12206.77	0.759

3.3.6 Robustness: The effect of minor intentional changes in the described chromatographic conditions is shown in Table 7. Robustness was performed to ensure the reliability of our method. The slight variations in the mobile phase flow rate and change in detection wavelength did not result in considerable differences in the analytes' retention time and peak area ratio. Thus, the described method exhibited robustness.

Table 7: ROBUSTNESS DATA OF OZ and CFX.

Parameter changed	Change in condition	Mean Peak Area ± SD (n=3)		RSD or %CV	
		OZ	CFX	OZ	CFX
pH	3.1 ± 0.02	342689 ± 1890.41	237486.6 ± 2132.41	0.552	0.898
	3.3 ± 0.02	342324 ± 1876.43	237825.6 ± 2168.14	0.548	0.912
Solvent co mposition	77:23 (Water:ACN)	342153 ± 1911.36	237813.4 ± 2152.61	0.559	0.905
	73:27 (Water:ACN)	342786 ± 1935.43	237825.6 ± 2149.12	0.565	0.904
Wave length	308	342786 ± 1844.36	237351.5 ± 2187.44	0.538	0.922
	312	342436 ± 1937.87	237825.6 ± 2106.13	0.566	0.886
Flow rate	0.9 mL/min	342875 ± 1843.89	237435.3 ± 2124.60	0.538	0.895
	1.1 mL/min	342563 ± 1906.18	237568.6 ± 2198.44	0.556	0.925

3.3.7 Specificity: The excipients present in the synthetic mixture during testing specificity do not interfere with the measurement of OZ and CFX. No additional peak interfering with the analyte-peaks was seen in the chromatogram Fig. 6A, 6B, 6C. Accordingly, our results suggested that the described analytical method demonstrated specificity.



6A: Bulk powder solution. 6B: Mobile phase. 6C: Sample Solution.

Fig 6: Chromatogram for Specificty test.

3.3.8 Solution stability

The stability test of OZ and CFX samples was performed by storing the API sample and test sample solution at 25°C and 2-8°C for 24 h and 72 hrs. The test was carried out in triplicate at each time point. The concentration results at various time points were not significantly different from one another. The result are displayed in Table 8.

Table 8: Stability of solutions.

Temp & Time	% Recovery ± SD (n=3)			
	API Sample		Test Sample	
	CFX	OZ	CFX	OZ
2-8 ° C 24 hrs	99.83 ± 0.25	99.85 ± 0.31	99.11 ± 0.36	99.42 ± 0.48
25° C 24 hrs	99.72 ± 0.33	99.81 ± 0.42	99.23 ± 0.46	99.38 ± 0.58
2-8 ° C 72 hrs	99. 75 ± 0.23	99.62 ± 0.21	98.87 ± 0.72	99.12 ± 0.45
25 ° C 24 hrs	99.65 ± 0.37	99.61 ± 0.33	99.02 ± 0.53	99.12 ± 0.41

Method Applicability: Assay of the Market Formulation

The proposed validated method was successfully applied to determine OZ and CFX in bulk powder and in tablet dosage forms. The results of the analysis of pharmaceutical dosage forms by the proposed method are highly reproducible, reliable and are in good agreement with the labeled claim of the drug. Results are given in Table 9. No interference of the excipients with the peaks of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of OZ and CFX in pharmaceutical dosage forms.

Table 9: Application of proposed RP-HPLC method to the determination in bulk and tablets.

Formulation	Drug	Labeled/taken amount (mg)	Amount found (mg)	% Amount found ± S.D. (n=5)
Bulk powder	Ornidazole	25	24.31	097.24 ± 1.53
	Cefixime	10	9.86	098.61 ± 1.23
Tablets	CEFLUV-OZ Ornidazole Cefixime	 500 200	 505.82 203.32	 100.17 ± 0.73 101.66 ± 1.54
	ZORNO Ornidazole Cefixime	 500 200	 501.04 195.12	 100.2 1± 0.72 097.56± 1.44

CONCLUSION

RP-HPLC method has been developed and validated for the simultaneous estimation of Cefixine and Ornidazole in bulk API mixture and tablet dosage form. The show that the method is accurate, precise, linear, robust, simple and rapid. Acceptable regression values,

%RSD and standard deviations which make it versatile and valuable for simultaneous estimation of two drugs in bulk and pharmaceutical dosage forms. The run time is relatively short. The results of this developed RP-HPLC method could be conveniently adopted for quality control analysis of Cefixine and ornidazole simultaneously from tablet dosage form.

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