ELECTROANALYTICAL STUDY FOR SIMULTANEOUS QUANTIFICATION OF CEFIXIME AND OFLOXACIN FROM PHARMACEUTICAL DOSAGE FORMULATION AND ITS DETERMINATION IN URINE.

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Abstract [(1- 2 & 5-7)]

In present study, a successful attempt has been made to develop a simple method for the simultaneous determination of cefixime and ofloxacin using Differential Pulse Polarography (DPP) technique. Quantification of cefixime and ofloxacin was done in Britton-Robinson Buffer of pH 5.0 using 1M KCl as a supporting electrolyte. Both cefixime and ofloxacin exhibit reduction cathodic peak in given pH with peak potential at -1.006 V for cefixime and -1.24 V for ofloxacin vs. S.C.E. 50% methanol-water solution was used as Solvent for the analysis. Proposed method was found to be simple, precise, and accurate and can be successfully applied for routine quality control analysis and simultaneous determination of cefixime and ofloxacin in combined drug formulation. The proposed method has been validated. The limit of detection and limit of quantification for cefixime in the standard solution was 7.39 μg/ml and 45.45 μg/ml while for ofloxacin in the standard solution was 4.98 μg/ml and 24.30 μg/ml respectively. The same validated method was successfully used in quantification of drugs in urine as well.

Keywords: Differential Pulse Polarography (DPP), cefixime, ofloxacin, Britton-Robinson Buffer, Quantification

Some abbreviations used in the paper:
CFI- cefixime
OF- ofloxacin

Introduction[10]

Cefixime with Ofloxacin belongs to a group of medications called 'cephalosporin antibiotics' primarily used for the treatment of a wide range of bacterial infections of the skin, ears, lungs, prostate and urinary tract. Besides this, it also treats typhoid fever and STDs (sexually transmitted diseases) like chlamydia and gonorrhoea. A bacterial infection happens when harmful bacteria enter inside the body and start multiplying. These harmful bacteria are responsible for producing toxins that can cause diseases like strep throat, urinary tract infection (UTI), bacterial food poisoning, gonorrhea, bacterial meningitis, cellulitis, Lyme disease, and tetanus.

Cefixime with Ofloxacin does not work against viral infections like flu and colds. Cefixime + Ofloxacin contains 'Cefixime' (cephalosporin antibiotic) and 'Ofloxacin' (fluoroquinolone antibiotic) that works by preventing the formation of the outer covering of the bacteria (cell wall) required for their survival. Cefixime + Ofloxacin prevents the release of a chemical messenger (mucopeptides) by the bacteria, thereby weakening and destroying the bacterial cell wall. Cefixime + Ofloxacin is a broad range antibiotic that works against a wide range of both gram-positive and gram-negative bacteria.

A literature surveys reveals few Chromatographic methods i.e. HPLC HPTLC, Derivative and Extractive spectrophotometric methods for the simultaneous determination of cefixime and ofloxacin. Very little attention has been paid to the use of electroanalytical methods. A literature survey has revealed cyclic voltammetry and D.C polarography methods for the determination of cefixime and ofloxacin individually, but its simultaneous determination by using Differential Pulse Polarography has not been reported.

In the topical countries like India, the major problems of health arise due to improper lifestyle, unhealthy environmental conditions, unhygienic and substandard food. Infections caused by the microorganisms like, fungi, protozoa, are most common. Drugs with antibiotic activity have been used in the treatment of the same. Cefixime with Ofloxacin is a combination of two antibiotics: Cefixime and Ofloxacin. Cefixime works by preventing the formation of the bacterial protective covering which is essential for the survival of the bacteria in the human body. Ofloxacin works by preventing the bacteria from reproducing and repairing themselves. Together, they treat your infection effectively.

MATERIALS AND METHODS (EXPERIMENTAL)

Introduction To Workstation [8-9]

Electrochemical workstation- PG STAT 30 with 663 VA Electrode stand (Metrohm)

It is made up of three electrode system namely-

1) Hanging Mercury Drop electrode (HMDE) as the working electrode

2) Saturated calomel electrode as the reference electrode

3) Platinum electrode as the counter electrode

The pH measurements were made with Euiptrances model No. 610.

Reagents

Standard cefixime with ofloxacin was obtained from local pharmaceutical company. All the solutions were prepared in double distilled water. All the reagents use were of AR grade. Britton-**Robinson buffer solutions**-[100ml of 0.04M H_3BO_4 + 0.04M H_3PO_4 + 0.04M CH_3COOH]. Further the desired value of pH (5.0) was adjusted with the addition of 0.2 M NaOH

General Procedure:

ANALYTICAL METHOD DEVELOPMENT

Preparation of Standard Solution

25 mg of standard cefixime and 50 mg of standard ofloxacin was accurately weighed and dissolved in minimum volume of 50% Methanol, kept it in sonicator for 5 mins and made up to a volume of 25 mL in standard flask to give stock solution (1000 µg/ml of cefixime and 2000 µg/ml of ofloxacin resp). Further all the standard solutions containing the mixture of cefixime and ofloxacin were prepared using this stock solution.

Proposed Voltammetric Method

Simultaneous behavior of CFI & OF was studied in Britton Robinson buffer of pH range 2-10.

An aliquot of $20cm^3$ made up of 18 mL Britton-Robinson Buffer adjusted to pH 5.0 by 1M NaOH + 2 mL of 0.1M KCl as a supporting electrolyte was placed in the dry and clean voltametric cell. Then it was purged with

highly pure nitrogen gas for 180s. A negatively directed DP scan between the potential of 0.0 V to -1.6 V Vs. S.C.E was applied. The operational parameters were as follows: 1] Scan rate- 15 mVs$^{-1.}$ 2] Pulse amplitude- 60 mV. After recording a voltammogram of blank, aliquots of (0.5ml) each of the required standard solutions was added from the standard stock solution. Resulted voltammograms were recorded under the optimum experimental conditions. Peak currents were recorded. Calibration curve was prepared by plotting peak current versus concentration of cefixime and ofloxacin applied.

Preparation of Sample Solution

Commercial brand-named Zifi-O 100 DT (FDC Ltd.) containing cefixime and ofloxacin in combination was procured. This brand contained a label claim of 100 mg of cefixime and 100 mg of ofloxacin per tablet. Ten tablets of each brand were weighed and powdered for the analysis. The powder equivalent to 0.745 g was accurately weighed, transferred quantitatively to 100 mL volumetric flask; then added 50% Methanol, kept it in sonicator for 5 mins the mixture was vortexed for 10mins, the solution was filtered through Whatman filter paper no. 41 and finally volume of the solution was made up to 100 mL with diluent. Voltammograms for the sample solutions were analyzed by the method described as above. Voltammograms were recorded under the optimum experimental conditions. The amount of cefixime and ofloxacin was calculated from resulting peak current values using already constructed calibration graph.

(For cefixime: y = 2.8464 x – 28.0231) and

(For ofloxacin: y = 3.4067 x + 226.6223)

ANALYTICAL METHOD VALIDATION [3-4]

System Suitability

System suitability tests are used to ensure reproducibility of the equipment. The test was carried out by recording voltammogram for cefixime and ofloxacin at working concentration i.e. at 83.33 μg/ml, and 166.67 μg/ml respectively with five replicates and the mean was used for the whole calculations. The % RSD was found to be 0.63 for cefixime and 0.88 for ofloxacin, which was acceptable as it is less than 2%.

Specificity

The specificity of method was confirmed by observing the polarograms of both the combined standard solution and the drug sample solutions. The polarograms obtained from the drugs sample solution were found to be identical to those obtained for standard solution. The addition of the standard solution to the drug sample solution did not change the characteristics of differential pulse polarograms. This gives the validity of method for the determination of both drugs from combined pharmaceutical formulation.

Linearity and Range

The linearity for cefixime and ofloxacin were observed simultaneously by addition of standard solution. A good linear dynamic range was achieved in the concentration ranges of 45.45-187.5 μg/ml for cefixime and 24.39 – 230 μg/ml for ofloxacin.

Linear working range was selected from the above and was found to be 45.45-115.38 μg/ml for cefixime and 90,91 – 230 μg/ml for ofloxacin

The calibration curves were constructed with concentration (C) against peak current (Ip). The slope, Intercept, regression equation and correlation coefficient for the Ornidazole was obtained is given in **(Table 1 and figure 1)**

Limit of Detection and Limit of Quantitation

The limit of detection (LOD) and the limit of quantification (LOQ) for CFI and OF were determined by signal to noise ratio of 3:1 and 10:1 respectively. The replicates for blank solution were recorded 20 times and the mean current value at the reduction potential of cefixime (i.e. at -1.006 V) and ofloxacin (i.e. at -1.24 V) was calculated. The concentration at which the peak current was found three times of mean blank current was taken as a limit of detection. And the concentration at which peak current was found to be ten times than the mean blank current was selected as limit of quantification.

The LOD and LOQ of cefixime were 7.39 μg/ml and 45.45 μg/ml. and ofloxacin was found to be 4.98 μg/ml and 24.39/ml respectively. **(Table 1 and Figure 3)**

Intraday and Interday Precision

The intra-day and inter-day precision was used to study the variability of the method. It was checked by recording the voltammograms of standard solutions of cefixime and ofloxacin i.e. whole concentration ranges (45.45-115.38 μg/ml for CFI and 90.91– 230.77 μg/ml for OF) both at intra-day (three times within 24 hour) and inter-day (two times each during 3 days intervals) to check the precision. The mean % RSD for intra-day and inter-day precision for cefixime found to be 0.79 % and 1.9 % and for ofloxacin it was 0.15 % and 0.7 %, respectively.

Assay

The developed Voltametric method was used for determination of cefixime and ofloxacin from a commercial brand of formulations. The sample working solutions were analyzed by the developed method described above. Voltammograms were recorded under the optimum experimental conditions. Resulting peak currents of cefixime and ofloxacin were measured and the amount of cefixime and ofloxacin calculated using already constructed calibration graph. Assay studies were carried out at three different levels i.e. lower, middle and higher level. The percentage assay at three different levels for Ornidazole was found to be from 98.00 % to 102.00 %. The results were shown in **(Table 2).**

Robustness

The robustness of the method was examined by the consistency of peak height and peak shape with the deliberately small changes in the experimental parameter. It is a measure of its capacity to retain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

To determine the robustness of the proposed method, the following variations were made in the analytical method-

1] Scan rate by ± 0.5 mVs^{-1}. 2] Pulse amplitude ± 1.0 mV

These parameters were deliberately changed one at a time and the effect of these changes on the assay studies was carried out. The proposed method was found to be robust.

Accuracy (Recovery)

The recovery was used to evaluate the accuracy of the method. Accuracy of the method was determined using the method of standard addition. A fixed volume of standard cefixime and ofloxacin solution was mixed with different concentrations of preanalyzed sample solutions and mixtures were analyzed by proposed method. The percent recovery was determined at different levels i.e. from 50% to 200% level. The results of recovery analysis for cefixime and ofloxacin are shown in **(Table 3)**

Determination of cefixime and ofloxacin in urine sample by spiking method

The urine sample collected for the study was fresh human specimen, which was spiked with different quantities of CFI and OF. To avoid interference and to increase concentration of analyte solid liquid extraction was carried out. For this purpose , Waters Bond Elute C18 cartridge of 500 mg of sorbent with 3 mL volume was used. 0.4 , 0.5 and 0.6 mL of the standard stock solution of concentration 500 μg/ml of cefixime and 500 μg/ml of ofloxacin was spiked in 49.6, 49.5 and 49.4 mL of the urine sample respectively, such that the effective concentration of CFI and OF in the urine was 4.0 , 5.0 and 6.0 μg/ml.

Steps involved in Solid phase extraction :

The cartridge packing was first conditioned by passing 3.0 mL of 100 % methanol. Then it was washed with 3.0 mL of water. To condition the SPE tube packing 3.0 mL of Britton-Robinson Buffer of pH 5.0 was pass through. After conditioning step, entire sample (50 mL) was loaded on the SPE packing by using volumetric pipette. The unretained material was washed off with 3.0 mL of water. The retained material was then eluted with 3.0 mL of Britton-Robinson Buffer of pH 5.0 and the eluted solution was collected in the fresh test tube. To the eluted solution 1.0 mL standard stock solution was added and diluted to 19 mL with buffer solution. The entire solution was used for polarographic analysis. For quantitative determination of CFI and OF from urine 1.0 mL of standard stock solution was added again in the same polarographic cell and polarogram was recorded.

Result And Discussion

In the present study quantification of cefixime and ofloxacin have been done from the formulations using Differential Pulse Polarography technique. The developed method was validated as per the ICH guidelines (Table 1-3). But before the method development and subsequent validation, optimization of the conditions for the analyte was done i.e. pH, supporting electrolyte and also the parameters i.e. 1] scan rate 2] Pulse amplitude has been studied. During optimization of the conditions, the Voltammetric response of cefixime and ofloxacin in different buffer solutions have been studied i.e. Acetate, Phosphate and Britton-Robinson Buffer. Britton-Robinson buffer was prepared by mixing 0.04 M Boric acid, 0.04M Phosphoric acid and 0.04M Glacial

acetic acid. Further pH was adjusted with 0,2 M NaOH. In the Britton-Robinson Buffer the whole pH range i.e. pH 2.0 to pH 10.0 has been studied.

As the pH was shifted from acidic to basic there is change in peak potential was observed. Finally, Britton-Robinson Buffer of pH 5.0 was chosen as the best, due to good separation of both the analytes, more uniform peak shape, less tailing, less broadening of peak, normal base line starts and regression analysis. The 1M KCl used as a supporting electrolyte. With KCl more uniform and sharper peaks were observed. Pulse amplitude of 60 mV was chosen as optimum as there is loss of resolution at high pulse amplitude. As the concentration of OF increases the slight negative shift in potential was observed whereas the increase in the concentration of CFI tends a positive shift in the potential.

The Differential Pulse voltammograms of cefixime and ofloxacin were recorded at various scan rates. A scan rate of 15 mVs^{-1} was chosen as a best for the analysis. The height of peak increase gradually with concentration of cefixime and ofloxacin and the response of peak current $\mathbf{i_p}$ as function of concentration is linear.

No significant interference was observed from excipients commonly used in the formulation i.e. glucose, sucrose, starch, magnesium stearate or talc powder.

Advantages of this Method

1. Rapid determination of CFI & OF from the tablet formulation.
2. Easy sample preparation, good reproducibility, cost effective.
3. Can be applied for the drug analysis in any form without any special pretreatment.
4. Simple, Selective, Accurate and Precise.
5. Interference of background matrix can be easily removed.
6. This method can be extensively used in clinical research which will open new avenues in the field of research

Conclusion

1. All the validation parameters were found to be satisfactory hence this method can be used for routine quality control analysis.
2. Detection limit for both cefixime and ofloxacin in urine sample was found to be more than that in a sample without urine.

ACKNOWLEDGEMENT

REFERENCES

1. P. V. Rege*, P. A. Sathe, V. S. Salvi- A Simple Electroanalytical Method (DPP) for Simultaneous Determination of Norfloxacin and Tinidazole from Combined Pharmaceutical Drug Formulation" International Journal of Chem Tech Research: Vol.3, No.3, July-Sept 2011,1213-1219

2. Shrotri. C. K*, P. A. Sathe, P. V. Rege - "A simple Electroanalytical method for estimation of Rosiglitazone Maleate and Metformin Hydrochloride individually from pharmaceutical formulation. International Journal of Pharma Research and Development-IJPRD, 2011; Vol 3(3):15; May 2011,115 – 121

3. ICH, Q2A, Validation of Analytical Procedure: Methodology, In. Proc.Int.Con. Harmonization, Geneva (1994).

4. ICH Q2B, Validation of Analytical Procedure: Methodology, In. Proc. Int. Con. Harmonization, Geneva (1996).

5. Wagh VM, Sathe PA, Rege PV. Simultaneous determination of Norfloxacin and Metronidazole in combined drug formulation by a simple electroanalytical technique. International Journal of Pharmaceutical Research and Development. 2013; 5(04):064-070.

6. Yáñez C, Bollo S, Núñez-Vergara LJ, Squella JA. Voltammetric determination of nitroimidazopyran drug candidate for the treatment of tuberculosis. Analytical Letters, 2001; 34(13):2335-2348.

7. V. M.Wagh*, P. A. Sathe, P. V. Rege, V. S. Salvi. " Simultaneous Determination of Norfloxacin and Tinidazole in combined Drug formulation by a Simple Electroanlytical Technique" International Journal of Pharma Research and Development-IJPRD, 2013; Vol 5(04):15; June 2013, 064 – 070.

8. H. Schmidt and M.von Stacklberg, Modern polarographic methods, Academic Press, New York, 1963, pp. 13-16.

9. A. M. Bond and R.J. O'Halloran, J. Electroanal. Chem. 68, 257 (1976).

10. Cefixime and Ofloxacin; https://en.wikipedia.org/wiki/Cefixime and https://en.wikipedia.org/wiki/Ofloxacin

Table 1: Method validation parameters for determination of CFI and OF

Parameters	Values	
	CEFIXIME	OFLOXACIN
System suitability (n=5) %RSD	0.63%	0.80%
Linearity range (µg/ml)	45.45-187.50 µg/ml	24.39-230.77 µg/ml
Slope (m) [a)]	2.822	3.326
Intercept(c) [a)]	24.5260	240.8905
Correlation coefficient (R^2)	0.9997	0.9998
LOD (µg/ml)	7.39 µg mL^{-1}	4.98 µg mL^{-1}
LOQ (µg/ml)	45.45 µg mL^{-1}	24.39 µg mL^{-1}
Intraday precision (n=5)	0.79%	1.9 %
Interday precision (n=5)	0.15 %	0.7 %
Assay	98% to 102%	98% to 102%
Recovery	98% to 102%	98% to 102%

a) Of the equation $y = mx + c$, where y is peak area, m is the slope, x is the Concentration and c is the intercept

Table 2: Results of assay studies for CFI and OF

Brand Name	Zifi-O 100 DT (FDC) Ltd	
	Cefixime	Ofloxacin
Labeled claim (mg)	100 mg	100 mg
Drug found in mg	98.54 mg	99.00 mg
% RSD (n=5)	1.32	0.31
% Assay	98.54%	99.00 %

Table 3: Results of recovery studies for LF and OZ

Standard	Level	Conc. Of std [μg/ml]	Conc. of std Found [μg/ml]	RSD (%) (n = 5)	Recovery (%)
Cefixime	50 %	23.8	23.98	1.42	100. 71%
	100%	45.5	45.00	1.12	99.01 %
	150%	65.2	64.23	1.45	98.53 %
				Mean	**99.42 %**
Ofloxacin	50 %	47.6	47.10	0.36	98.91 %
	100%	90.9	89.29	0.13	98.22 %
	150%	130.4	131.18	0.29	100.57 %
				Mean	**99.24 %**
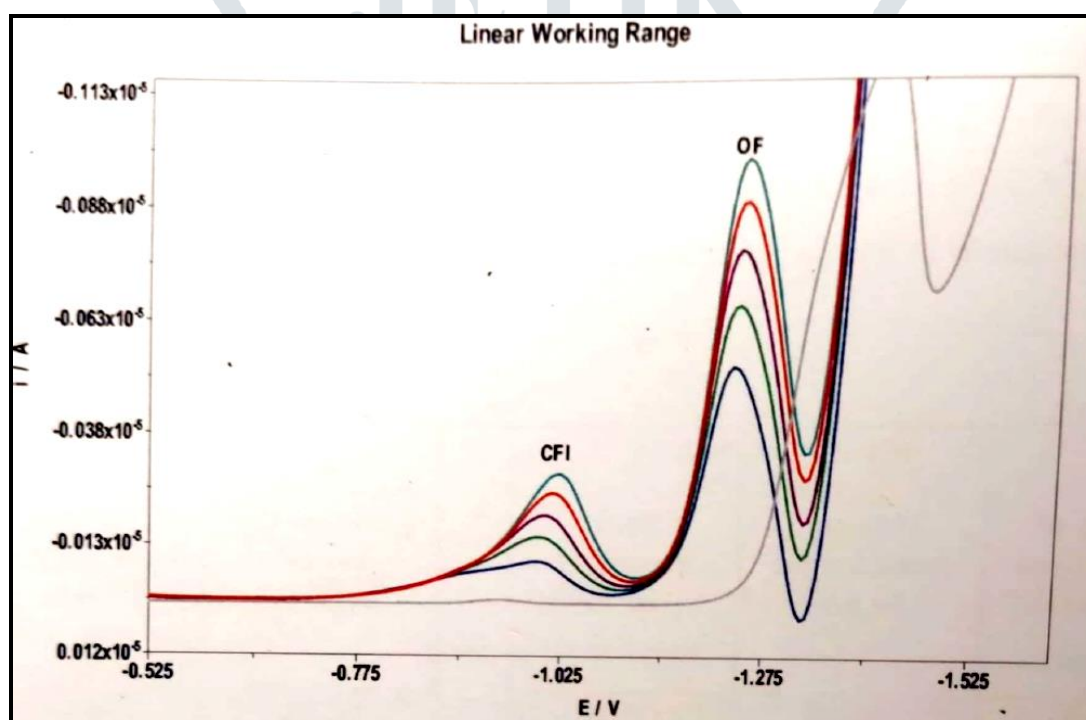
Figure-1. Voltammogram of CFI and OF
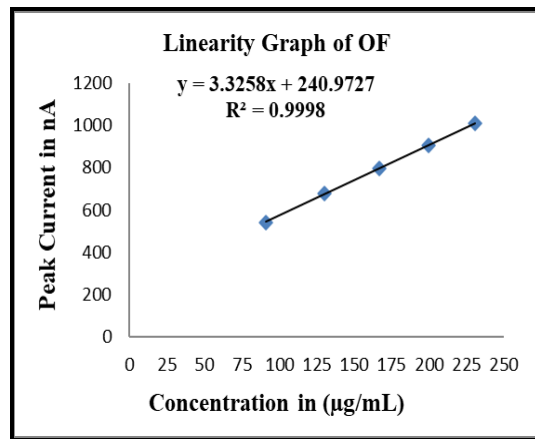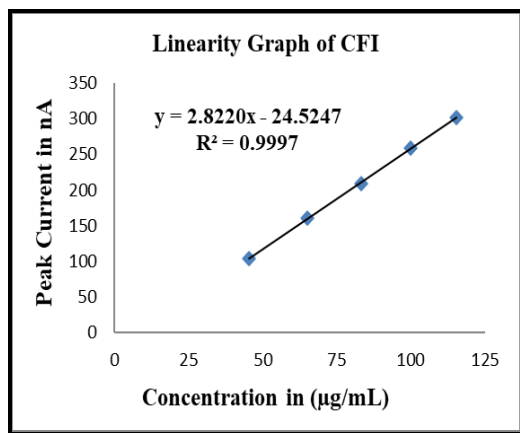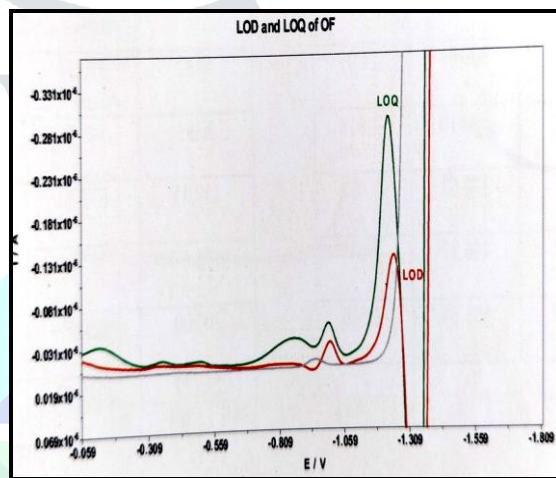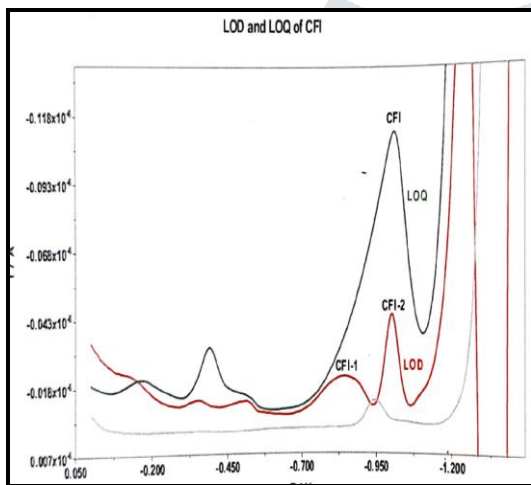
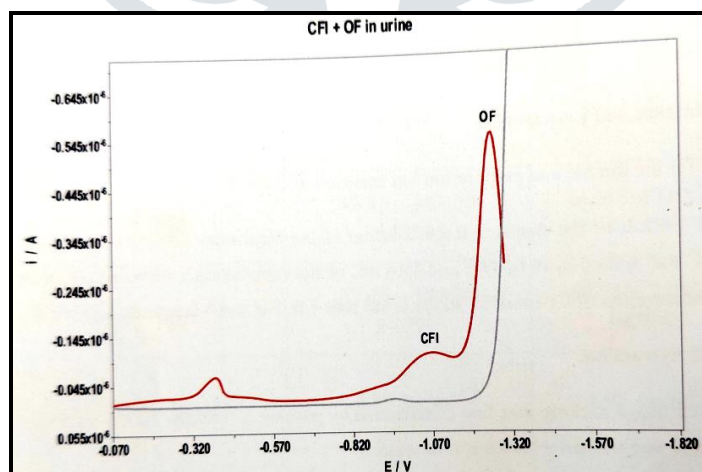**Figure-2. Linearity Graphs For**

Figure-3. LOD & LOQ graphs for cefixime & ofloxacin

Figure-4. Graph for CFI + OF in Urine