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Research Article

Development And Validation Of RP- HPTLC Method For Simultaneous Estimation Antibacterial Drugs from Pharmaceutical Formulation

Pralhad Rege*[1], Avinash Jagdale[2] and Niraj Bahujuni[3]

*[1]*Assistant Professor & Research guide, Dept of Chemistry, St. Xavier's College, Mumbai*
[2]*Research Scholar, Dept of Chemistry, St. Xavier's College, Mumbai*
[3]*Lecturer, Dept of Chemistry, St. Xavier's College, Mumbai*

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ABSTRACT

In present study, a successful attempt was made to develop a simple, sensitive, precise and validated densitometric RP-HPTLC method for the simultaneous determination of Ofloxacin and Cefixime from combined pharmaceutical drug formulation. Chromatographic separation was achieved on aluminium plates pre-coated with silica gel 60F254 as the stationary phase. The solvent system consisted of n- butanol: methanol: water: formic acid (8:6:4:0.3 v/v) which was found to give compact and dense spots for OFX and CFX with Rf value 0.17 ± 0.02 and 0.50 ± 0.02 respectively. Densitometric analysis of both drugs were carried out in the absorbance mode at 293 nm. The developed method was validated and proved to meet the requirements delineated by ICH guidelines with respect to linearity, accuracy, precision, specificity and robustness. The regression analysis data for the calibration plots showed a good linear relationship with correlation coefficient greater than 0.999 for both ofloxacin and cefixime in the concentration range of 100-300 ng/band. The LOD and LOQ for OFX were found to be 15.0 and 45.45 ng/spot respectively and for CFX , 16.16 and 50.4 ng/spot respectively. Assay results for tablet formulation were found to be 99.30 ± 0.96 % and 100.8 ± 0.98 % of label claim for OFX and CFX respectively. The validated method was successfully applied for determination of both the drugs in their pharmaceutical formulation indicating the ability of proposed method to be used for routine quality control analysis of these drugs.

INTRODUCTION

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 the most common. Drugs with antifungal and antiprotozoal activity have been used in the treatment of the

***Corresponding Author:** Dr. Pralhad Rege
Address: *Dept of Chemistry, St. Xavier's College*
Email : pralhad1806@gmail.com

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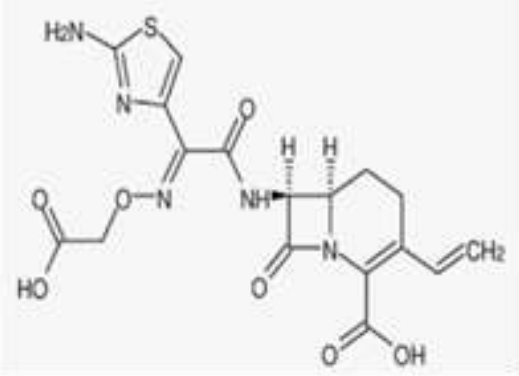
same. In many cases, drugs with two active ingredients are prescribed to the patients to have an added advantage. Many of these antibacterial drugs are found in combination with antifungal and antiprotozoal drugs which are highly effective against fungal and protozoal infections. **Cefixime**, C16H15N5O7S2 that is (6R,7R)-7-[[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2 (carboxymethoxyimino)acetyl]amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2 carboxylic acid. (Molecular weight:- 453.5 g/mol)] is used in the treatment of bacterial infection. Cefixime is a broad-spectrum, third-generation cephalosporin antibiotic derived semi synthetically from the marine fungus Cephalosporium acremonium with antibacterial activity. **Ofloxacin**, C18H20FN3O4 that is 7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-azatricyclo[7.3.1.05,13]trideca-5(13),6,8,11-tetraene-11-carboxylicacid; hydrochloride is second generation fluroquinolone with an antibacterial spectrum of activity used as an antibacterial drug. (Molecular weight: - 361.4 g/mol) **Ofloxacin** is a quinolone antibiotic useful for the treatment of a number of bacterial infections. A literature survey has revealed very few chromatographic and spectrophotometric methods. In the present work we have focused on deciding the optimum chromatographic conditions for the simultaneous determination of Cefixime and Ofloxacin in combined pharmaceutical drug formulations and successfully developed completely novel validated method for the same.

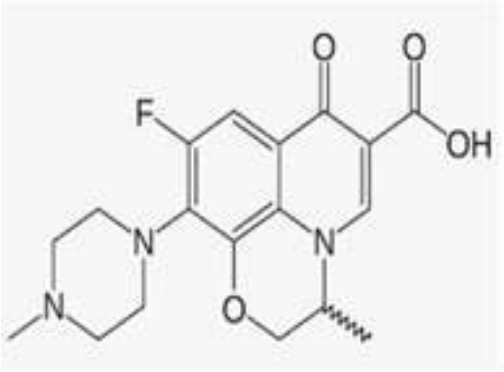
OBJECTIVE

The main objective of study is to provide a simple, rapid, efficient, reliable and economic method for the simultaneous determination of Cefixime and Ofloxacin in combined pharmaceutical formulations using RP- HPTLC. The proposed developed method to be subsequently validated as per ICH guidelines.

STRUCTURE



Cefixime



Ofloxacin

MATERIAL AND METHODS

CHEMICALS AND REAGENTS

Standard Cefixime and Cloxacillin were obtained from local pharmaceutical company with claimed purity above 99.0%. All the solutions were prepared in double distilled water. All the necessary reagents used i.e. water and methanol (HPLC grade). Mobile phase was filtered using 0.45µm syringe filter made by Millipore whereas; Whatman's filter paper No.41 (purchased from local market) was used in the preparation of sample solution

APPARATUS AND CHROMATOGRAPHIC CONDITIONS

Sample Applicator: CAMAG automatic TLC sampler 4 (ATS 4) (100 µl Syringe)

Development chamber: CAMAG Twin Trough chamber (TTC)

Densitometric scanning: CAMAG TLC Scanner 4 (Vision CATS software 3.1)

Chromatographic Mode	Gradient
Stationary phase	TLC silica gel 60 F254, Aluminum plate, 20 x 10 cm (60 pore size, Merck 5554)
Mobile Phase	n- butanol: methanol: water: formic acid (8:6:4:0.3 v/v)
Application Volume	2 µl
Separation technique	Ascending
Chamber saturation	20 minutes (Mobile phase)
RH (%)	50 +/- 5
Flow rate	2.0 ml/min
Temperature (°C)	25 +/- 1
Wavelength:	293 nm for Cefixime and Ofloxacin
Scanning mode	Reflectance/Absorbance
Slit Dimension	6 x 0.45 mm
Rf Value for Cefixime	0.50
Rf Value for Ofloxacin	0.17

PRAPARATION OF STANDARD SOLUTION

Weigh accurately 20 mg of Cefixime standard and 20 mg Ofloxacin standard transfer it into a 20 ml standard flask, shake well to mix and make up to the volume with Methanol: water (80:20). The working standard solution 0.1 mg/mL of Cefixime and 0.1 mg/mL of Ofloxacin were prepared by diluting 2 ml of this solution in to a 20 ml standard flask, mix and dilute up to the volume with Methanol: water (80:20).

PRAPARATION OF SAMPLE SOLUTION

Commercial brand (Zifi-O) containing of Cefixime and Ofloxacin in combination was procured. Brand sample contained a label claim of 200 mg of Cefixime and 200 mg of Ofloxacin per tablet. Ten tablets were weighed and powdered for the analysis. The sample powder (748mg) equivalent to 200 mg of Cefixime and 200 mg of Ofloxacin was accurately weighed, transferred into 100ml standard flask; add 70 ml of Methanol: water (80:20) and sonicate to dissolve. Allow it to cool at room temperature, mix well and the mixture was sonicated for 30 mins, finally volume of the solution was made up to 100 mL with Methanol: water (80:20). The solution was filtered through 0.45µm syringe filter made by Millipore and appropriate volume (i.e. 2.0 mL) of stock solution was diluted to 20 mL with the diluent to obtain a solution containing 200 ng/band of Cefixime and 200 ng/band of Ofloxacin, correspondingly were employed for the quantitative analysis.

ANALYTICAL METHOD VALIDATION

SPECIFICITY

The specificity of method was confirmed by observing the chromatograms of both the combined standard solution and the drug sample solutions. The chromatograms 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 chromatograms. This gives the validity of method for the determination of both the drugs from combined pharmaceutical formulation.

LINEARITY AND RANGE

The linearity for Cefixime and Ofloxacin was observed simultaneously by addition of standard solution. Linearity of the method was studied by spotting five concentrations of each drug prepared in the methanol: water (80:20), in the range of 100-300 (ng/band) and 100-300 (ng/band) for Cefixime and Ofloxacin respectively and noted the peak area responses. The calibration curves were constructed with concentration (C) against peak area. The slope, intercept, regression equation and correlation coefficient for the CFX and CLX was obtained is given in **Table 1 and Figure 4**

LOD AND LOQ

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula;

$$LOD = \frac{3.3 \times \text{S. D of the response}}{\text{Slope of calibration curve}}$$

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified. LOQ was calculated using the following formula;

$$LOQ = \frac{10 \times \text{S. D of the response}}{\text{Slope of calibration curve}}$$

LOD and LOQ for Cefixime were 16.6 ng/band and 50.4 ng/band and for Ofloxacin were found to be 15.0 ng/band and 45.5 ng/band respectively.

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 chromatograms of sample solutions of Cefixime and Ofloxacin at working level i.e. 100% both at intra-day (six times within 24 hour) and inter-day (six times during 3 days intervals) to check the precision. The mean % RSD for intra-day and inter-day precision was found to be less than 1.0% for both CFX and CLX. Result of intra and inter day precision studies are given in **Table 1**

ASSAY

The developed chromatographic method was used for simultaneous determination of Cefixime and Ofloxacin from commercial brand of formulation. The sample solutions were analyzed by the developed method described above. Chromatograms were recorded under the optimum experimental conditions. Resulting peak area of Cefixime and Ofloxacin were measured and the amount of Cefixime and Ofloxacin calculated using already constructed calibration graph. Result of assay studies is given 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. Robustness of the method was performed by intentionally modifying the chromatographic conditions such as composition of mobile phase, change in flow rate and change in oven temperature. In the robustness study, the influence of small, deliberate variations of the analytical parameters on retention time of the drugs was examined. The following factor was selected for change: Chamber saturation time: 30 minutes instead of 20 minutes (Mobile phase) and mobile phase composition (± 0.1 mL). The solution containing 200 ng/spot of Cefixime and Cloxacillin was applied onto the column. A number of replicate analyses (n = 3) were conducted for evaluation of each change of factors. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPTLC method developed is robust. Result of robustness studies is given in **Table 4 and Figure 3**

ACCURACY (RECOVERY)

The recovery was used to evaluate the accuracy of the method. Accuracy of the method was determined using the method of varying weight of sample for sample preparation. A weight of sample was varied at different concentrations of preanalyzed sample solutions and analyzed by proposed method. The percentage recovery was determined at different levels i.e. from 50% to 150% level. The results of recovery analysis for Cefixime and Cloxacillin are shown in **(Table.3)**.

RESULT AND DUSCUSSION

HPTLC is a useful technique for the resolution and, in turn for the determination of drug mixtures. This technique offers a simple way to quantify

drugs directly on TLC plate by measuring the optical density of the separated bands. The amounts of compounds are determined by comparing to a standard curve from reference materials chromatographed simultaneously under the same conditions. In the present work conditions were optimized for development and validation of a simple and accurate RPHPTLC method for simultaneous quantification of Cefixime and Ofloxacin in combined pharmaceutical drug formulation. Method development was right from optimization of the condition and parameters i.e., selection of system, column, mobile phase, different composition of mobile phases have been tried. Use of pre-coated silica gel HPTLC plates with n- butanol: methanol: water: formic acid (8:6:4:0.3 v/v) resulted in good separation of the drug. The proposed method for two drugs gave well defined peaks. They were well separated too. The correlation of coefficient (r2) obtained was 0.9997 for both Cefixime and Ofloxacin, that means a good linear relationship was observed between the concentration range 100 to 300 µg/spot for both the drugs. The system suitability experiment was carried out before the determination of CFX and OFX unknown sample. The coefficient of variation was less than 2% for replicate measurements of the same sample. This shows that the method and the system both are suitable for the determination of unknown samples. The assay of CFX and OFX was found to be between 98 % to 102 % range. The accuracy and reproducibility of the proposed method was confirmed by recovery experiments. Recovery studies were found in the range 98 % to 102 % which indicates high accuracy of the method. The absence of additional peaks in chromatogram indicates non- interference of the common excipients used in tablet formulation. The percentage recovery obtained indicates non-interference from the excipients used in the formulation. The reproducibility, repeatability and accuracy of the proposed method were found to be satisfactory which is evidenced by low values of percentage relative standard deviation in comparison to previous methods. The low values of LOD and LOQ indicate the high sensitivity of the method. As the proposed method is highly accurate, selective and precise hence can be used for simultaneous determination of Cefixime and Ofloxacin in pharmaceutical preparations. The method is also relatively fast and requires approximately 45 min for the analysis. Results for regression analysis, assay, recovery and robustness studies are given in Table 1, 2, 3 and 4 respectively.

CONCLUSION

In addition to above mentioned points, the proposed method is found to be more simple, economic, accurate and practical. Thus, presented method can be recommended for simultaneous determination of Cefixime and Ofloxacin in routine quality control analysis in combined drug formulations.

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REFERENCE

1. Raj,-SV; Kapadia,-SU; Argekar,-AP Simultaneous determination of metronidazole and norfloxacin from pharmaceutical preparations by RP-HPLC. Indian-Drugs. Oct 1997; 34(10): 585-589
2. Kumar R. Siva., Nallasivan P. Kumar., Saravanakumar S., Kandasamy C.S., and Venkatnarayanan R., "Simultaneous RP-HPLC Estimation of Nitazoxanide and Cloxacillin in Tablet Dosage Forms", Asian J. Research Chem. 2(1): 2009 Jan.-March, , pg. 43-45.
3. Paramane S., Kothapalli L., Thomas A., Deshpande A.D., "Simultaneous RP-HPLC

estimation of Gemifloxacin and Ornidazole in Tablet Dosage Form", Indian Journal of Pharmaceutical Sciences, 2007, July - August, pg. 525-528.

4. F. Havaldar, A. Aggarwal and P. Rege: "Stability indicating RP-HPLC method for simultaneous determination of beclomethasone dipropionate and clotrimazole from dosage form" International Journal of Chem Tech Research, Vol.10 No.9, pp 437-442, 2017.
5. https://en.wikipedia.org/wiki/Cefixime
6. https://en.wikipedia.org/wiki/Ofloxacin
7. ICH, Q2A, Validation of Analytical Procedure: Methodology, In. Proc.Int.Con. Harmonization, Geneva (1994).
8. ICH Q2B, Validation of Analytical Procedure: Methodology, In. Proc. Int. Con. Harmonization, Geneva (1996)
9. Argekar,-AP; Kapadia,-SU; Raj,- SV Simultaneous determination of norfloxacin and tinidazole in pharmaceutical preparations by high-performance thin-layer chromatography. J-Planar-Chromatogr-Mod-TLC. May-Jun 1996; 9(3): 208-211
10. Ganhimathi M; Ravi T.K; and Shukla N. Validated High-Performance Thin Layer Chromatography method for simultaneous estimation of Cloxacillin and Ornidazole, Indian J. Pharm. Sci.; 68, 2006, pg. 838-840.
11. Rashmin, P.; Mrunali, P.; Nitin, D.; Nidhi, D.; Phara, P. T. HPTLC method development and validation: strategy to minimize methodological failures. J. Food Drug Anal. 2012, 20, 794–804.
12. Bhole, R. P.; Shinde, S. S.; Chitlange, S. S.; Wankhede, S. B. High performance thin layer chromatographic method for simultaneous determination of diphenhydramine hydrochloride and naproxen sodium in tablets. Anal. Chem. Insights 2015, 10, 47–51.
13. Mahajan, K. S.; Chauhan, R. S.; Shah, S. A.; Shah, D. R. Development of HPTLC method for simultaneous estimation of paracetamol and flupirtine maleate in their combined tablet dosage form. J. Pharm. Appl. Sci. 2015, 2(1), 1–7.
14. Shah, D.; Bhatt, K.; Mehta, R.; Baldania, S.; Gandhi, T. RPHPLC determination of Atorvastatin Calcium and Amlodipine Besylate combination in tablet. Ind. J. Pharm. Sci. 2006, 68, 796–799.

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Table. 1: Method validation parameters for the determination of Cefixime and Ofloxacin

Parameters	Values	
	Cefixime	**Ofloxacin**
Linearity range (µg/mL)	100 to 300 ng/band	100 to 300 ng/band
Slope (m) [a)]	0.0001	0.0001
Intercept(c) [a)]	-0.00034	-0.00124
Correlation coefficient (R^2)	0.9997	0.9997
LOD (µg/mL)	15.0 ng/band	16.6 ng/band
LOQ (µg/mL)	45.5 ng/band	50.4 ng/band
Intraday precision (n=6)	1.3 %	1.02 %
Interday precision (n=6)	0.9 %	1.01%
Assay	99.9% to 100.7%	98.7% to 99.4%
Recovery	98.5% to 99.5%	98.6% to 101.3%

Sample Details

Brand Name: ZIFI-O (FDC LIMITED)

Batch No.: 010I049

API: Ofloxacin-200 mg and Cefixime-200 mg

Excipients: q.s.

Colour: Tartrazine

Table 2: Result of Assay studies of Cefixime and Ofloxacin

Brand name	ZIFI-O (FDC Limited)	
	Ofloxacin	**Cefixime**
Labeled claim (mg)	200mg	200mg
Drug found in mg	199.6 mg	201.5 mg
% RSD (n=3)	0.72	0.82
% Assay	99.8 %	100.5 %

Table 3: Results of Recovery studies of Cefixime and Ofloxacin

Analyte	Level	Weight of Sample taken (mg)	Sample Conc. In accuracy study (in µg/mL)	Amount found in recovery study (in mg/ tablet)	RSD (%) (n = 3)	Recovery (%)
Cefixime	**50%**	374.5	50	202.5	0.86	101.25
	100%	747.9	100	201.1	0.50	100.55
	150%	1127.2	150	202.0	0.38	101
						100.93
Ofloxacin	**50%**	374.5	50	199.8	0.57	99.9
	100%	747.9	100	201.6	0.72	100.8
	150%	1127.2	150	201.7	0.98	100.85
						100.51

Table 4: Results for Robustness study

Sr. No.	Component	Rf value
1	Cefixime standard	0.52
2	Ofloxacin standard	0.17

3	Sample (For Cefixime and Ofloxacin)	For Cefixime: 0.52
		For Ofloxacin: 0.17

Figure-1. HPTLC Densitometric scan of Cefixime and Ofloxacin

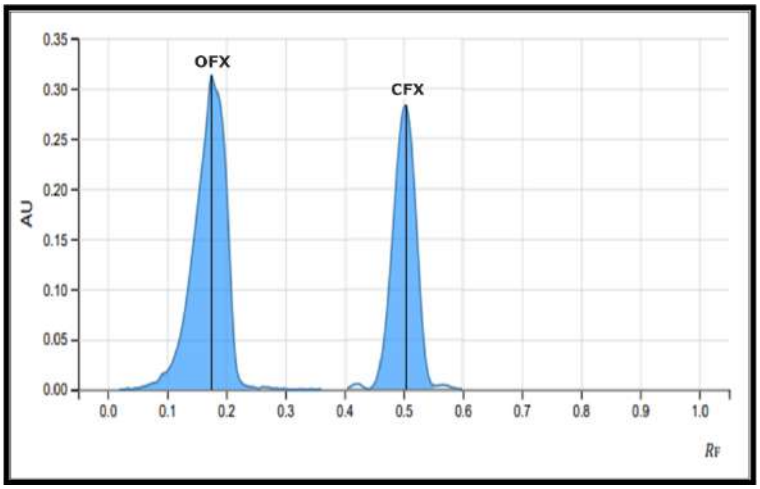


Figure 2 HPTLC plate image of Cefixime and Ofloxacin

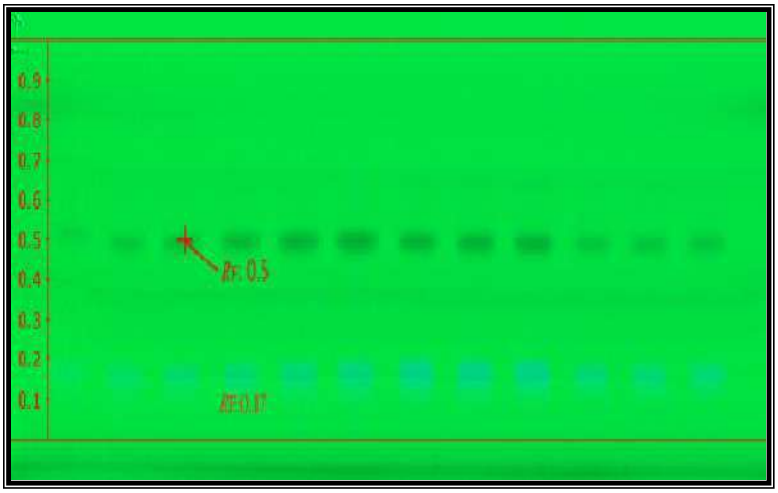


Figure 3 HPTLC plate image of Cefixime and Ofloxacin for Robustness study

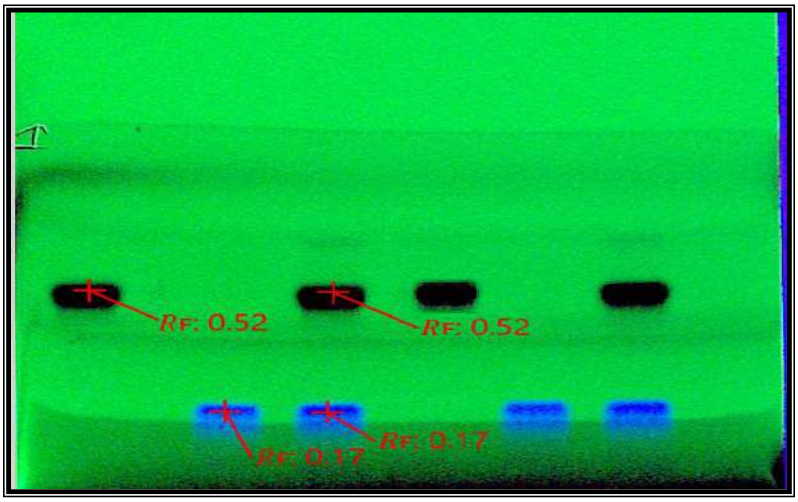
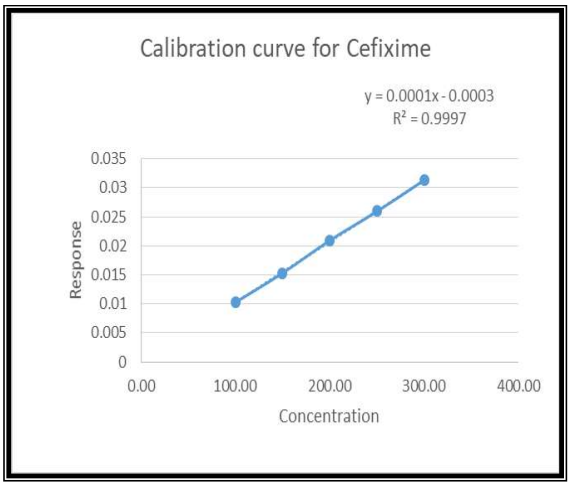
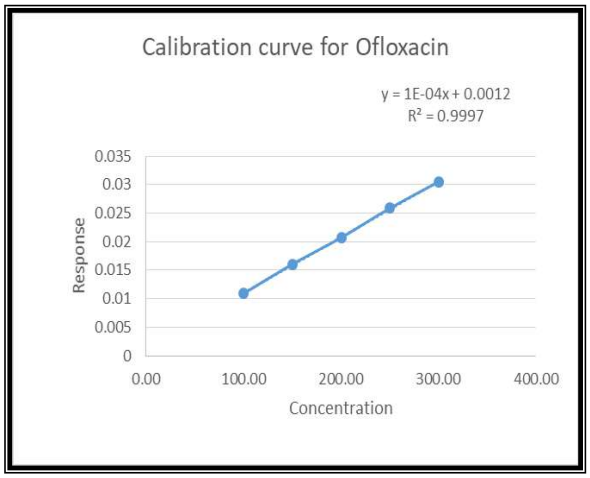


Figure-4. Linearity graph for

Cefixime Standard



Ofloxacin Standard



Y-axis – Peak Area

X-axis- Concentration of Drug in µg/mL