Research Article

DEVELOPMENT AND VALIDATION OF THE RP-HPLC METHOD FOR THE ESTIMATION OF CEFPODOXIME AND DICLOXACILLIN IN THEIR COMBINED DOSAGE FORM AND ITS APPLICATION TO THE DISSOLUTION STUDY

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ABSTRACT

A Reverse Phase Liquid Chromatography (RP-HPLC) for the simultaneous estimation of Cefpodoxime (Cefpo) and Dicloxacillin (Diclox) in their combined dosage form was developed and validated and the above developed RP-HPLC method was applied to the Dissolution study. The method was performed on a RP-HPLC (YL-Clarity System), Kromasil ODS C_{18} (5 µm) column, 250×4.6 mm, 5µm particle size. The detection was carried out at 235 nm using Acetonitrile: Water (70:30 v/v) as a mobile phase at a flow rate of 1 ml/min at ambient temperature. For Dissolution Study, Cefpodoxime and Dicloxacillin ER tablet was kept in 0.1 N HCl for 2 hrs then the tablet was kept in Phosphate buffer 6.8 pH for 24 hrs. Sampling was carried out from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24 hrs. Linearity was observed in concentration range of 5–25 µg/ml and a correlation coefficient of 0.999 for Cefpodoxime and in concentration range of 12.5-62.5 µg/ml and a correlation coefficient of 0.9993 for Dicloxacillin respectively. For Dissolution Study, ZEDOCEF-DXL-200 (Cefpodoxime and Dicloxacillin ER Tablets) showed mean % cumulative release of 87.588 ± 2.1078 and 92.16 ± 1.8757 of labeled amount of Cefpodoxime and Dicloxacillin Extended release tablets within 24 hours respectively. The RP-HPLC method was found to be simple, sensitive, precise and reproducible. The developed RP-HPLC method could be used for dissolution study of extended release tablet dosage form of Cefpodoxime and Dicloxacillin successfully.

Keywords: RP-HPLC, Cefpodoxime, Dicloxacillin.

INTRODUCTION

Cefpodoxime is (6*R*,7R)-7-{[(2*Z*)-2-(2-amino-1,3-thiazol-4-yl)-2-methoxyimino-acetyl]amino}-3-(methoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (Figure 1) an oral third generation cephalosporin antibiotic. It is commonly used to treat acute otitis media, pharyngitis and sinusitis.[1, 2]

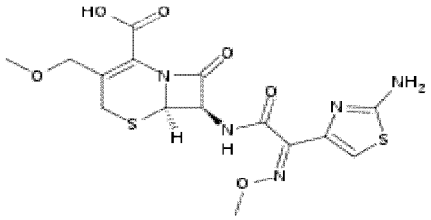

Figure 1: Chemical Structure of Cefpodoxime

Dicloxacillin is (2*S*,5*R*,6*R*)-6-{[3-(2,6-dichlorophenyl)-5-methyl- oxazole-4 carbonyl]amino}-3,3-dimethyl-7-oxo-4-thia- 1-azabicyclo [3.2.0] heptane-2-carboxylic acid a narrow-spectrum beta-lactum antibiotic of the penicillin class. It is commonly used in Pneumonia, septic arthritis and throat infections.[3, 4]

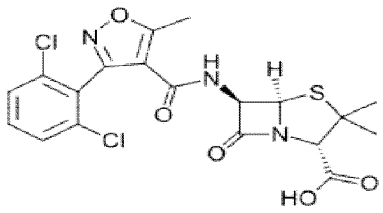

Figure 2: Chemical Structure of Dicloxacillin

Cefpo is official in Indian Pharmacopoeia and US Pharmacopeia[2, 5] while Diclox is official in US Pharmacopeia and British Pharmacopei.[6, 7]. Literature survey reveals that numbers of analytical methods are reported for the estimation of Cefpo and Diclox in single and combined dosage forms. Reported methods for estimation of Cefpo are Spectrophotometric methods [8-10], Spectrofluorimetric method [11], HPLC method [12, 13]and HPTLC method[14] and similarly Reported methods for estimation of Diclox are Spectrophotometric method [15], Second-derivative Spectrophotometric method [16], RP-HPLC methods [17-19].

No Method has been reported for estimation of Cefpodoxime and Dicloxacillin by RP-HPLC in combined dosage form & there is also no method has been reported for its application to the dissolution method. So the rational of work is to develop and validate simple, accurate and precise RP-HPLC method for the estimation of these two drugs in their combined dosage form and its application to the Dissolution Study.

MATERIALS AND METHODS

Apparatus and Instruments

- HPLC: Make & Model: Young - Linn Clarity 9100 HPLC System

Degasser: Vacuum Degasser YL – 9101

Pump: Quaternary Pump YL – 9110

Detector: PDA detector YL – 9160

Column: Kromasil ODS C_{18} (5 µm) column, 250×4.6 mm

Temperature: Ambient

Pressure: 1000 - 3000 psi

- Double beam UV-visible spectrophotometer (Shimadzu, Model 1800) having two matched quartz cells with 1 cm light path
- Electronic analytical balance, Shimadzu AUX-220
- Ultra sonicator
- Digital pH Meter (JankiImpex Pvt. Ltd., Ahmedabad)
- Dissolution Apparatus: PLC Dissolution Rate Test Apparatus U.S.P/B.P/I.P. STD
- Borosilicate Volumetric flask – 10, 25, 50, 100 ml
- Borosilicate Pipettes – 1, 2, 5, 10 ml
- All instruments and glass wares were calibrated.

Reagents and Standards

- Standard Cefpodoxime Proxetil (gift sample from Sunrise Remedies Pvt. Ltd. Ahmedabad)
- Standard Dicloxacillin Sodium (gift sample from Rhombus Pharma Pvt. Ltd. Ahmedabad)
- Combined tablet formulations (Zedocef-DXL-200) were procured from Indian market.
- Acetonitrile for Chromatography Lichrosolv® (Merck Specialities Pvt. Ltd., Mumbai)
- Water for Chromatography Lichrosolv® (Merck Specialities Pvt. Ltd., Mumbai)
- Sartorius Filter Paper 0.2 micron (Sartorius, Germany)
- 0.1N HCl
- 0.2M NaOH
- 0.2 M Potassium dihydrogen phosphate

Method

Selection of Analytical Wavelength

For Selection of wavelength for HPLC determination, two same concentration solutions of both drugs i.e. Cefpo and Diclox of 10µg/ml were prepared. UV spectrums of both drugs were taken and then both spectrums were overlapped. From overlapping of spectras (Figure 6.1), the wavelength at where both spectra's were cross each other was taken as wavelength for HPLC determination. For Cefpo and Diclox, 235 nm was taken for HPLC determination.

Preparation of Standard Solutions

Preparation of Cefpo standard solution

Accurately weighed 50 mg of Cefpo was transferred into 50 ml volumetric flask and dissolved in 30 ml Optimized mobile phase as a diluent. The flask was sonicated for 5 min. The flask was shaken and volume was made up to the mark with diluent to give a solution having strength of 1 mg/ml. (1000 µg/ml).

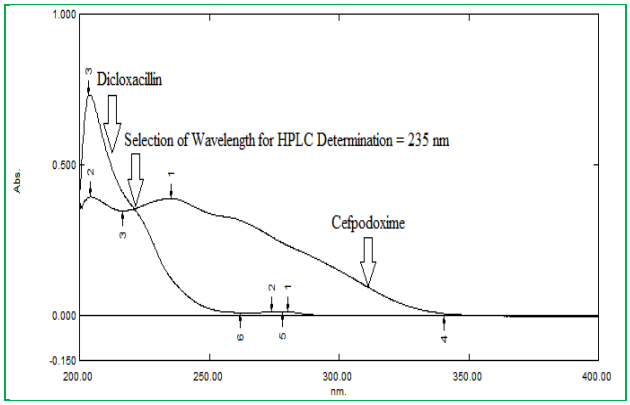

Figure 3: UV spectra of standard solution of Cefpodoxime (10µg/ml) and Dicloxacillin (10µg/ml) for selection of detection wavelength.

Preparation of working standard solution of Cefpodoxime

Pipette out 1 ml of stock solution (1000 µg/ml) and transfer into 10 ml volumetric flask and make up to the mark with diluent to prepare 100 µg/ml.

Preparation of Dicloxacillin standard stock solution

Accurately weighed 50 mg of Diclox was transferred into 50 ml volumetric flask and dissolved in 30 ml Optimized mobile phase as a diluent. The flask was sonicated for 5 min. The flask was shaken and volume was made up to the mark with diluent to give a solution having strength of 1 mg/ml. (1000 µg/ml)

Preparation of working standard solution of Dicloxacillin

Pipette out 1 ml of stock solution (1000 µg/ml) and transfer into 10 ml volumetric flask and make up to the mark with diluent to prepare 100 µg/ml.

Preparation of Dissolution Medium 0.1N HCl

59.5 ml of 36% pure concentrated HCl was added to distilled water to prepare10 liters of 0.1NHCL.

Preparation of Dissolution Medium (Phosphate Buffer 6.8 pH)

1.875 liter of 0.2 M Potassium dihydrogen phosphate and 840 ml of 0.2 M NaOH were added to distilled water to prepare 7.5 liters of phosphate buffer 6.8 pH.

Chromatographic Condition

HPLC Model: YL9100 HPLC, Young Lin Instrument

Stationary Phase: Kromasil ODS C_{18} (5 µm) column, 250×4.6 mm

Mobile Phase: Acetonitrile: Water (70:30 v/v)
Flow rate: 1 ml/min

Detection Wave length: 235 nm

Temperature: Ambient

Run time: 8 minutes

Injection volume: 20 µl

Preparation of Calibration Curve

Appropriate aliquots from Cefpo standard stock solution (1000 µg/ml) were transferred in separate 10 ml volumetric flasks and volume made up to the mark with mobile phase (Acetonitrile: Water :: 70:30 v/v) to obtain final concentration of 5, 10, 15, 20 and 25 µg/ml. Appropriate aliquots from Diclox standard stock solution (1000 µg/ml) ware taken in separate 10 ml volumetric flasks and volume made up to the mark with mobile phase (Acetonitrile: Water :: 70:30 v/v) to obtain final concentration of 12.5, 25, 37.5, 50, 62.5µg/ml. The solutions were injected using a 20 µl loop system and chromatograms were recorded. Then, calibration curves were constructed by plotting peak area vs. concentration of the drug and regression equations were computed for Cefpo and Diclox respectively.

System Suitability Test

Observed values of Resolution, Column efficiency, Tailing factor were depicted in Table 1.

Table 1: System Suitability Test Parameter

System Suitability Parameters	Proposed Method		Standard Values
	Cefpo	Diclox	
Retention times (Rt)	3.607	1.727	-
Theoretical plates (N)	3242	2218	Greater than 2000
Resolution (Rs)	7.830		Greater than 2
Tailing factor (As)	0.916	1.558	Not greater than 2.0

Analytical Method Validation

Validation of developed method was carried out as per ICH Q_2 R_1 guideline.[20] Parameters such as Linearity, Accuracy, Precision, LOD, LOQ, Ruggedness and Robustness were taken up as tests for analytical method validation.

Linearity and Range

The proposed RP-HPLC method shows good linearity in the concentration range of 5 to 25µg/ml for Cefpo and 12.5 to 62.5 µg/ml for Diclox depicted in Figure 4 and 5. Overlay Chromatograms of standard mixture of Cefpo and Diclox depicted in Figure 6.

Precision

The intraday precision of the developed method was evaluated by analyzing combined samples of different concentrations of Cefpo and Diclox three times on the same day and %RSD was calculated.

The inter day precision was evaluated from the combined concentration of Cefpo and Diclox on three different days and %RSD was calculated. The repeatability was evaluated by combined standard solutions of Cefpo (15 µg/ml) and Diclox (37.5 µg/ml) were prepared and analyzed six time on the same day. Results obtained are shown in Table 2.

Table 2: Summary of Validation Parameters

Validation Parameters	Cefpo	Diclox	Standard Values
Linearity (conc. range)	5-25µg/ml	12.5-62.5 µg/ml	-
Regression equation	Y = 47.411x – 4.1441	Y = 26.627x + 48.508	-
Correlation coefficient (r^2)	0.999	0.9993	≥ 0.999
Precision (%RSD)			
Repeatability Intraday Interday	0.55 0.34 1.02	1.20 0.44 1.17	≤ 2.0 %RSD
LOD (µg/ml)	0.53	0.35	
LOQ (µg/ml)	1.62	1.08	
% Recovery	100.28	100.65	98 – 102%
Specificity	Specific		
Ruggedness	Complies		≤ 2.0 %RSD
Robustness: Changing in Flow rate Changing in Mobile phase ratio Changing in Detection Wavelength	Complies		≤ 2.0 %RSD

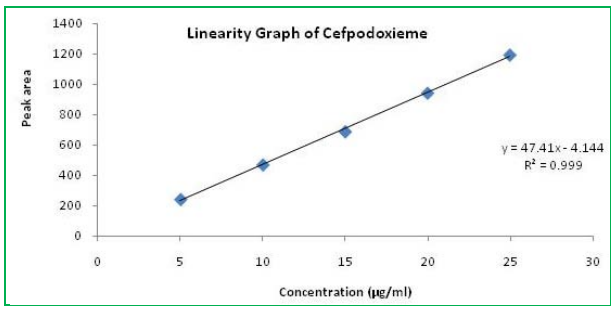

Figure 4: Linearity graph of Cefpodoxime

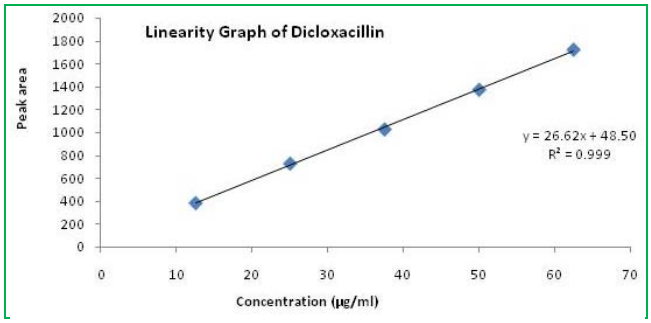

Figure 5: Linearity graph of Dicloxacillin

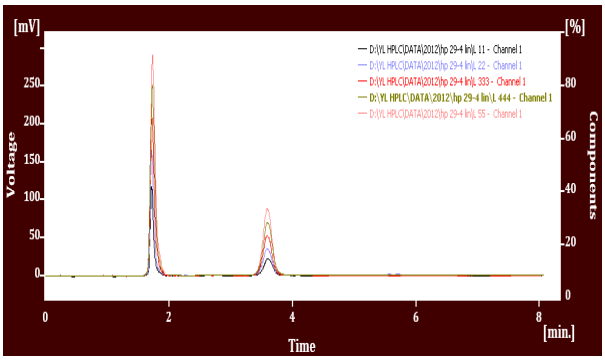

Figure 6: Overlay Chromatogram of Cefpodoxime (5-25 µg/ml) and Dicloxacilin (12.5-62.5 µg/ml)

Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition from 50 % to 150 % of label claim. The results are shown in Table 3 and 4. Recovery greater than 98% with low SD justifies the accuracy of the method.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. Typically, these might include impurities, degrades etc. A solution of placebo in mobile phase was injected and the chromatogram showed no inferring peaks at retention time of the two drugs. The chromatogram of placebo were compared with those acquired from Cefpo and Diclox standards, correlation was good (in terms of t_R and area) indicates the specificity of method. Chromatograms of specificity for Cefpo and Diclox depicted in Figure 8 to 10.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Calibration curve of mixture was repeated for 5 times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated as follows.

LOD=3.3 * SD/slope of calibration curve,

LOQ=10 * SD/slope of calibration curve.

Where, SD = Standard deviation of intercepts

Results obtained are shown in Table 2.

Ruggedness

Ruggedness of the proposed method was determined by analysis of aliquots of sample solution (15 µg/ml Cefpo and 37.5 µg/ml Diclox) by two analyst using same operational and environmental conditions. Results obtained are shown in Table 5.

Table 3: Recovery of Cefpodoxime from Formulation

Amount taken (µg)	Amount added (µg)	Total amount of Cefpo (µg)	Amount of Cefpo recovered (µg ± SD)(n=3)	% Mean Recovery of Cefpo
7.5	3.75	11.25	11.292 ±0.02622	100.37
7.5	7.5	15	14.975 ± 0.06649	99.83
7.5	11.25	18.75	18.87 ± 0.08245	100.64
Average % Mean Recovery				**100.28**

Table 4: Recovery of Dicloxacillin from Formulation

Amount taken (µg)	Amount added (µg)	Total amount of Diclox (µg)	Amount of Diclox recovered (µg ± SD)(n=3)	% Mean Recovery of Diclox
18.75	9.375	28.125	28.252 ±0.16059	100.45
18.75	18.75	37.5	37.763 ± 0.40585	100.70
18.75	28.125	46.875	47.240 ± 0.17457	100.78
Average % Mean Recovery				**100.65**

Table 5: Ruggedness Data

Ruggedness Study by Analyst – I		
Analyst - I	**Cefpo**	**Diclox**
Mean % Assay* ± SD	99.346±0.32562	100.433±0.23028
% RSD	0.32776	0.22929
Ruggedness Study by Analyst – II		
Analyst - II	**Cefpo**	**Diclox**
Mean % Assay* ± SD	99.596±0.76552	99.863±0.81867
% RSD	0.76862	0.81857

Table 6: Robustness results for variations in Method Parameters

Method Parameter	Mean (n = 3)		SD		% RSD	
	Cefpo	Diclox	Cefpo	Diclox	Cefpo	Diclox
Wavelength (nm)	99.957	98.8663	0.91166	0.91572	0.91205	0.92622
% of Acetonitrile (v/v)	99.752	99.5823	0.71826	1.10952	0.72004	1.11418
Detection Wavelength	100.209	99.5703	0.84035	1.09496	0.83859	1.09969

Table 7: Analysis of market formulation

Formulation	Labeled amount (mg)		% Assay (n = 6)	
	Cefpo	Diclox	Cefpo	Diclox
Tablet	200	500	99.48	100.02

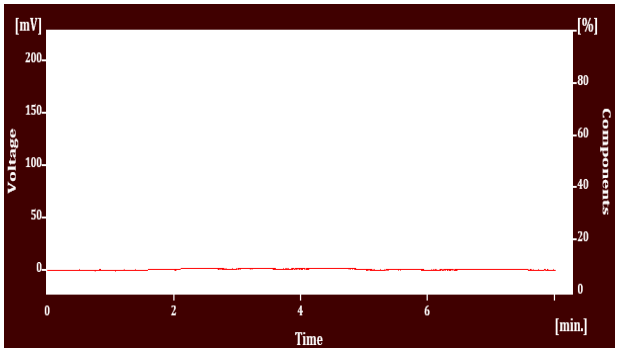

Figure 7: Specificity Chromatogram of Blank Placebo in Mobile Phase

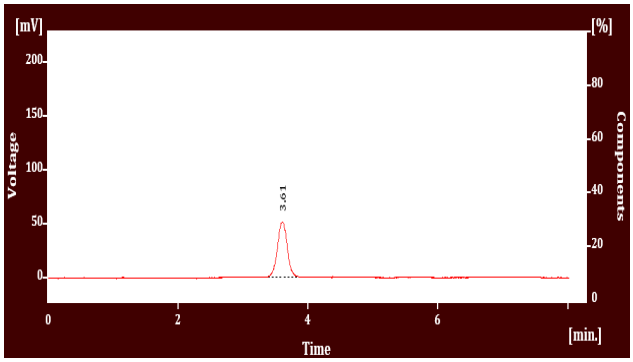

Figure 8: Specificity Chromatogram of Cefpodoxime (15 µg/ml)

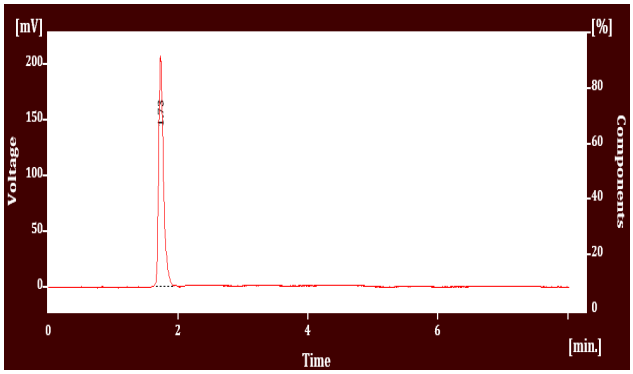

Figure 9: Specificity Chromatogram of Dicloxacillin (37.5 µg/ml)

Robustness

The Robustness of the method was evaluated by

- By changing the flow rate by 1.0 ± 0.1 ml/min (0.9 ml/min and 1.1 ml/min)
- By changing mobile phase ratio by 70 ± 1.0 % (69 and 71 %) for Acetonitrile
- By changing detection wavelength by 235 ± 2 nm (233 nm and 237 nm)

Results are shown in Table 6.

Analysis of Marketed Formulation by Proposed Method

Twenty tablets were weighed and average weight of content was determined & the content of tablets was powdered. The powder equivalent to 15 mg of Cefpo or 37.5 mg of Diclox was transferred in to a 100 ml volumetric flask, dissolved and diluted up to the mark with mobile phase as a diluent. The solution was filtered through Sartorius filter paper (0.2 µ). An aliquots of 1 ml of this solution was diluted to 10 ml with mobile phase six times.

Each solution was injected using Rhenodyne Injector (Fixed Capacity Loop of 20 µl) and chromatograms were recorded. The peak area of each chromatogram was determined. The concentration of each drug was calculated using calibration curve equation.

The results are shown in Table 7.

Application of the above developed RP-HPLC method to the Dissolution Study

Procedure for Dissolution Study

1000ml of dissolution medium (0.1N HCl) was transferred into each vessel of dissolution apparatus. The paddles were lowered up to the specified position. Then rpm was set to 50 and temperature to 37±0.5°C. The tablet was placed at each of the bottom of vessels and peddle was operated.

10ml of sample was withdrawn from each of the vessel at 0, 0.5, 1, 2 hours from the zone midway between the surface of the dissolution medium and the top of paddle, not less than 100 mm from the wall of the vessel.

Replace the medium with drawn for analysis with equal volume of fresh medium.

Each Sample which was withdrawn from the 0.1 N HCl Dissolution medium was neutralized with 0.1 N NaOH before it was measured by HPLC.

After 2 hour, Tablet was replaced in Phosphate Buffer 6.8 pH within 10 minutes and dissolution conditions were set as it were set in 0.1 N HCl.

10 ml of sample was withdrawn from each of the vessel at 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24 hours from the zone midway between the surface of the dissolution medium and the top of paddle, not less than 100 mm from the wall of the vessel.

The withdrawn medium for analysis was replaced with equal volume of fresh medium. Samples were measured by HPLC. Standard solutions were prepared containing mixture of Cefpo (200 µg/ml) and Diclox (500 µg/ml) respectively.

Percentage drug release was calculated by comparing peak area of different samples which were taken at different interval with peak area obtain by Standard solution containing mixture of Cefpo (200 µg/ml) and Diclox (500 µg/ml) respectively. Mean % release of Cefpo and Diclox are shown in table 8. Comparison of % Release of Cefpodoxime and Dicloxacillin is shown in the figure 10.

Table 8: Mean % release of Cefpodoxime and Dicloxacillin

Time (hr)	% Release ± SD of Drugs	
	Cefpodoxime	Dicloxacillin
0	0.00	0.00
0.5	2.05 ± 0.1756	3.93 ± 0.3285
1	3.09 ± 0.2479	4.09 ± 0.1321
2	5.99 ± 0.4470	7.48 ± 0.3633
3	30.36 ± 0.9780	33.35 ± 0.7564
4	35.27 ± 1.0734	37.12 ± 0.8330
5	42.91 ± 0.7796	43.93 ± 1.1055
6	52.54 ± 0.9641	48.16 ± 1.0987
7	59.51 ± 204082	52.08 ± 1.2211
8	63.68 ± 1.2386	55.44 ± 1.3113
9	67.58 ± 1.3597	58.32 ± 2.1951
10	69.56 ± 1.0905	63.54 ± 1.2571
11	73.73 ± 1.6052	72.44 ± 1.7165
12	75.87 ± 1.5156	74.23 ± 1.8644
24	87.588 ± 2.1078	92.16 ± 1.8757

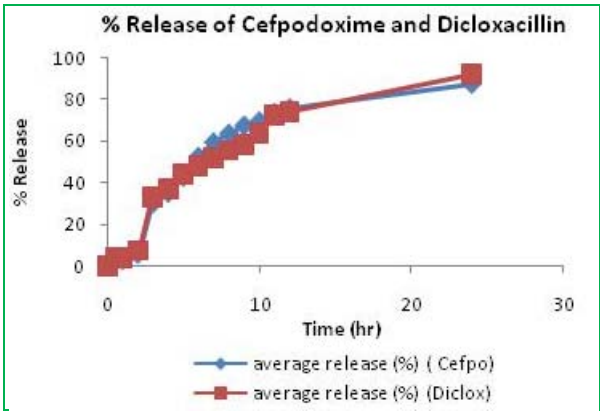

Figure 10: % Release of Cefpodoxime and Dicloxacillin

ZEDOCEF-DXL-200 (Cefpo and Diclox ER Tablets) show mean % cumulative release of 87.588 ± 2.1078 and 92.16 ± 1.8757 of labeled amount of Cefpo and Diclox Extended release tablets within 24 hours respectively.

RESULTS AND DISCUSSION

A simple, specific, accurate and precise RP-HPLC method has been developed and validated for simultaneous estimation of both these drugs. The chromatographic separation was achieved on Kromasil ODS C_{18} (5 µm) column, 250×4.6 mm using Acetonitrile: Water (70: 30 v/v) as mobile phase at 235 nm. RP-HPLC method shows linearity in the range of 5-25 µg/ml for Cefpo and 12.5-62.5 µg/ml for Diclox. The correlation coefficient was 0.999 and 0.9993 was found for Cefpo and Diclox respectively. The average percentage recoveries of Cefpo and Diclox for RP-HPLC method are of 100.28% and 100.65% respectively. The average percentage assay results of Cefpo and Diclox for RP-HPLC method are of 99.48% and 100.02% respectively. This is comparable to labeled claim. System suitability test reveal that all system suitability parameters complies with standard values. ZEDOCEF-DXL-200 (Cefpo and Diclox ER Tablets) show mean % cumulative release of 87.588 ± 2.1078 and 92.16 ± 1.8757 of labeled amount of Cefpo and Diclox Extended release tablets within 24 hours respectively.

CONCLUSION

We have successfully developed a new simple RP-HPLC method for the simultaneous estimation of Cefpo and Diclox combination in mixture using simple mobile phase acetonitrile and water. This method could be used for analyses, including pure drug analysis, assay of formulations and stability studies analysis. The purposed method did not utilize any extraction step for recovering the drug from the formulation excipient matrixes and their by decreased the degree of error, time in estimation of the drugs and the overall cost of the analysis. The method was validated and found to be simple, sensitive, accurate, precise and economical. The proposed method could be applied for routine analysis in quality control laboratories. From dissolution study, it is concluded that Dicloxacillin release faster than Cefpodoxime.

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