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DEVELOPMENT AND VALIDATION OF AN RP-HPLC METHOD FOR DETERMINATION OF CEFIXIME AND DICLOXACILLIN IN TABLET DOSAGE FORM

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Abstract

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for simultaneous estimation of Cefixime and Dicloxacillin in bulk drug and tablet dosage form. Sepration was achieved by capcell pack C-18 column having 250 mm× 4.6 mm i.d. in isocratic mode, with mobile phase containing 0.05 M potassium dihydrogen phosphate: methanol (25:75), adjusted to pH 4.5 using ortho phosphoric acid. The flow rate was 1.0 ml/min and effluents were monitored at 232 nm. The retention time of Cefixime and Dicloxacillin were 3.19 min and 6.68 min respectively. The linearity for Cefixime and Dicloxacillin were in the range of 10-100 µg/ml. The recoveries of Cefixime and Dicloxacillin were found in the range of 99.63-99.95 % and 99.58-99.98 % respectively. The proposed method was validated as per ICH and USP guidelines and successfully applied to the estimation of Cefixime and Dicloxacillin in bulk drug and tablet dosage form.

***Keywords*:** Cefixime, Dicloxacillin, RP-HPLC Method, Validation

1. Introduction

Analytical chemistry is the analysis of material samples to gain an understanding of their chemical composition and structure. Analytical chemistry is the science of making quantitative measurements. In practice, quantifying analytes in a complex sample becomes an exercise in problem solving. To be effective and efficient, analyzing samples requires expertise in the chemistry that can occur in a sample analysis and sample handling methods for a wide variety of problems (the tools-of-the-trade) proper data analysis and record keeping.[1,2] Analytical chemistry requires broad background knowledge of chemical and physical concepts. These hypermedia documents contain links to the fundamental principles that underly the different analytical methods. With a fundamental understanding of analytical methods, a scientist faced with a difficult analytical problem can apply the most appropriate technique(s). A fundamental understanding also makes it easier to identify when a particular problem cannot be solved by traditional methods and gives an analyst the knowledge that is needed to develop creative approaches or new analytical methods.[3] Analytical chemistry is the study of the chemical composition of natural and artificial materials. Properties studied in analytical chemistry include geometric features such as molecular morphologies and distributions of species, as well as features such as composition and species identity. Unlike the sub disciplines inorganic chemistry and organic chemistry, analytical chemistry (like physical chemistry) is not restricted to any particular type of chemical compound or reaction. The contributions made by analytical chemists have played critical roles in the sciences ranging from the development of concepts and theories (pure science) to a variety of practical applications, such as biomedical applications, environmental monitoring, quality control of industrial manufacturing and forensic science (applied science).[4]

2. Materials and Instruments

Drug sample suppliers & Manufacturers:

Table 1: Drug sample suppliers & Manufacturers:

S.No	Name of Drugs	Drug supplies & Manufacturers.
1	Cefixime	Emcure Pharmaceutical Ltd. Pune
2	Dicloxacillin	Aurobindo Pharma Ltd. Hydrabad

List of Reagents and Chemicals used:

Table 2: List of Reagents and Chemicals used:

Name of chemicals	Suppliers
Ortho- phosphoric acid	Research Lab Fine Chem. Industries
Methanol AR grade	Merk Ltd., Mumbai
Glacial actic acid HPLC grade	Research Lab Fine Chem Industries, Mumbai.
Acetonitrile HPLC grade	Merk Specialities Pvt. Ltd., Mumbai
Methanol HPLC grade	Thomas Baker (chemicals) Pvt. Ltd., Mumbai

Instrument used:

Table 3: Instrument used:

Equipments	Company
AUX220 electronic balance	Shimadzu corporation, Japan
V-530 UV-Visible double beam spectrophotometer	JASCO corporation, Japan
Cyberlab HPLC system 2003 LC-P-100 HPLC pump Rheodyne manual sample injector Capcell pack C-18 (4.6mm×250mm) column LC-UV-100 as UV-VIS detector	Cyberlab corporation, Japan

Corresponding Author*: prabhupharma1@gmail.com

Softwares used: Shanupro.

2.1 Methodology

2.1. 1. Determination of λ_{max} of Cefixime:

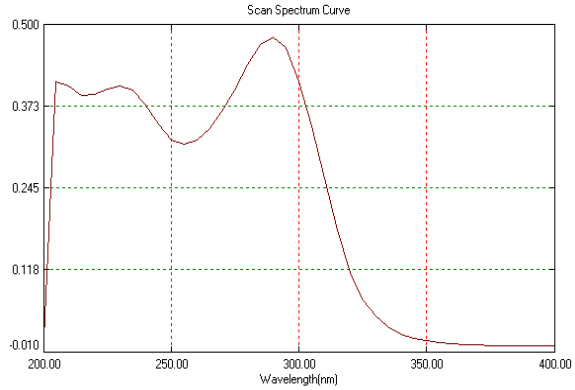


Fig.1: UV spectra of Cefixime

The standard solution of Cefixime was scanned at different concentrations in the range of 200-400 nm and the λ_{max} was found to be 290 nm against reagent blank.

2.1.2. Determination of λ_{max} of Dicloxacillin:

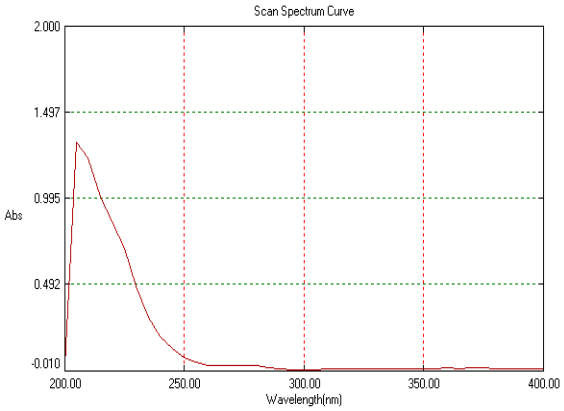


Fig. 2: UV spectra of Dicloxacillin

The standard solution of Dicloxacillin was scanned at different concentrations in the range of 200-400 nm and the λ_{max} was found to be 210 nm against reagent blank

2.2 RP-HPLC Method for Cefixime and Dicloxacillin.

Determination of Cefixime and Dicloxacillin in combined tablet dosage form.

Method 1. Solubility of drugs in different solvents:

Solubility of both drugs was observed by dissolving them in different solvents.

Table. 4: Solubility of drugs in different solvents:

Solvents	Solubility	
	Cefixime	Dicloxacillin
ACN	+	+
Water	+	+
Methanol	++	++

(+++) Symbolizes highly soluble, (++) Symbolizes freely soluble, (+) Symbolizes soluble, (-) Symbolize insoluble.

Method 2:

A. Selection of analytical wavelength:

By appropiate dilution of each standard stock solution with mobile phase, various concentrations of Cefixime and Dicloxacillin were prepared separately .Each solution was scanned in between the range of 200 nm to400 nm and their spectra were overlaid. The wavelength selected for the analysis was 232 nm which both the drugs showed significant absorbance. The overlain UV spectra of Cefixime and Dicloxacillin in the mobile phase are as shown in figure below.

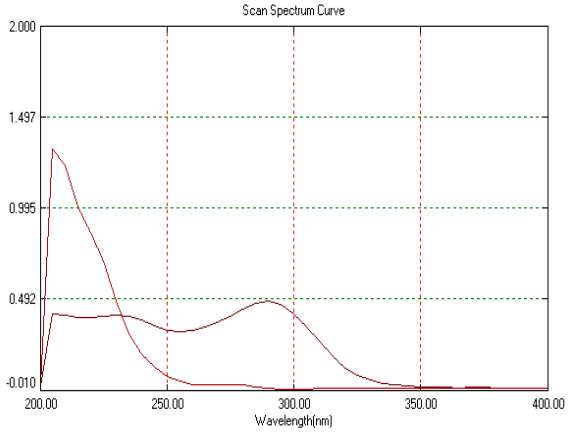


Fig. 3: Overlain spectra of Cefixime and Dicloxacillin

B. Calibration of Cefixime & Dicloxacillin:

Take 10 mg of Cefixime & 10 mg of Dicloxacillin seperately in 100 mL volumetric flask which was then dissolved in 50 mL mobile phase with shaking. Then it was sonicated and the volume was made up to 100 mL. Suitable dilutions are made from the standard stock solutions. The linearity of the relationship between peak area and concentration was determined by analyzing six working standards over the concentration range of 10, 20, 30, 40, 50 and 100µg/mL for Cefixime & 10, 20, 30, 40, 50 and 100µg/mL for Dicloxacillin. Hence the peak areas were recorded against concentration & graph of concentration Vs peak area was plotted.

Fig. 4: Chromatogram of 10 µg/mL of Cefixime

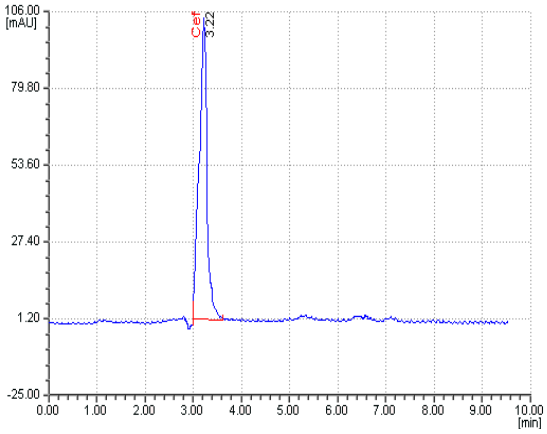


Fig. 5: Chromatogram of 20 µg/mL of Cefixime

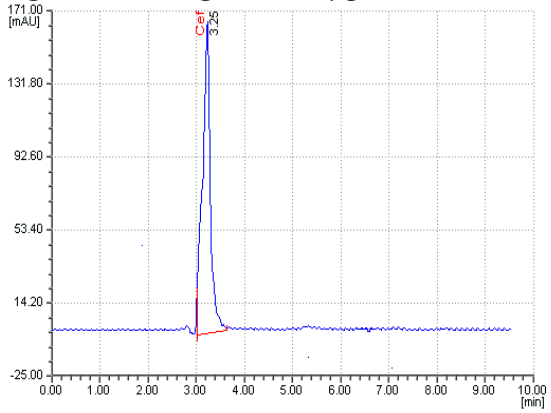


Fig. 6: Chromatogram of 30 µg/mL of Cefixime

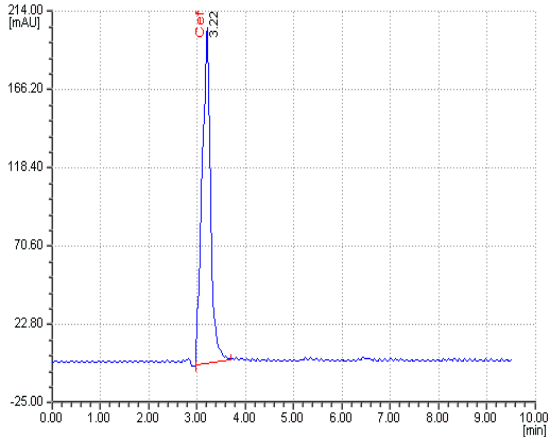


Fig. 7: Chromatogram of 40 µg/mL of Cefixime

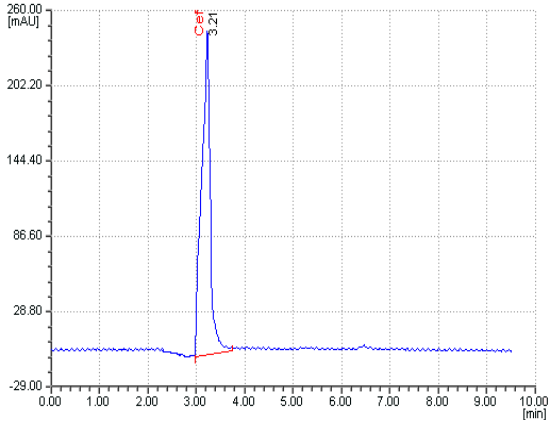


Fig. 8: Chromatogram of 50 µg/mL of Cefixime

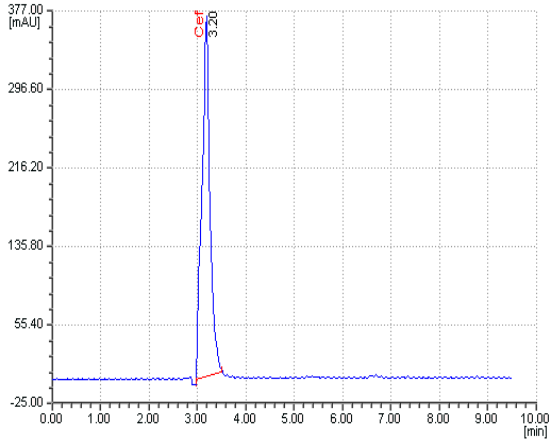


Fig. 9: Chromatogram of 100 µg/mL of cefixime

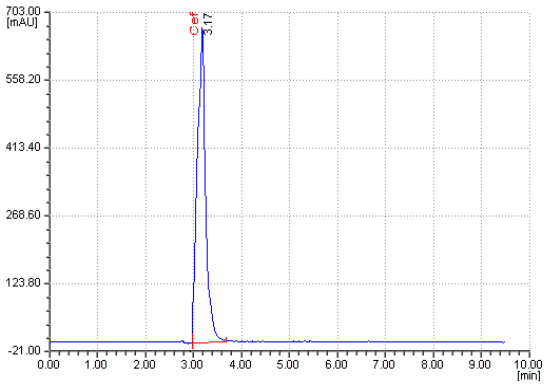


Fig. 10: Calibration curve of Cefixime

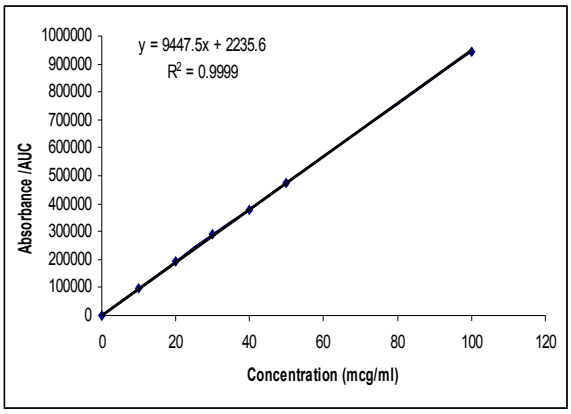


Fig. 11: Chromatogram of 10 µg/mL of Dicloxacillin

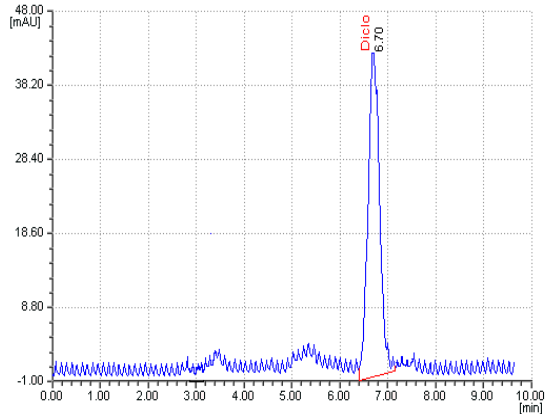


Fig. 12: Chromatogram of 20 µg/mL of Dicloxacillin

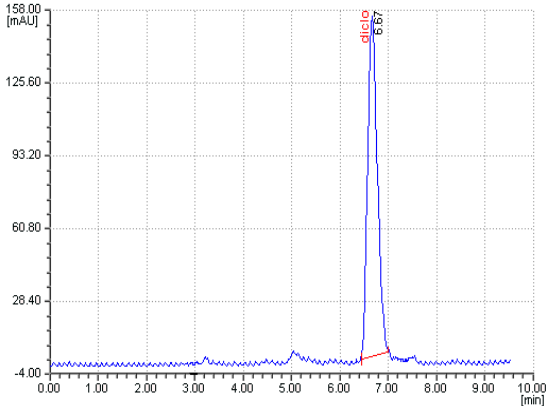


Fig. 13: Chromatogram of 30 µg/mL of Dicloxacillin

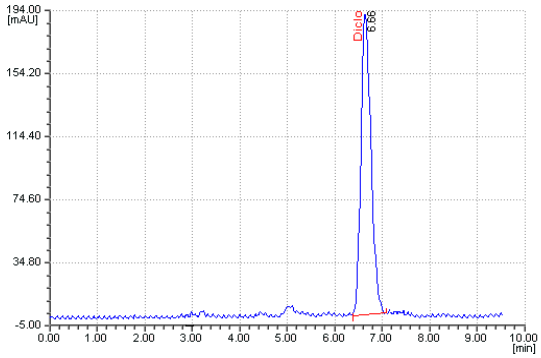


Fig. 14: Chromatogram of 40 µg/mL of Dicloxacillin

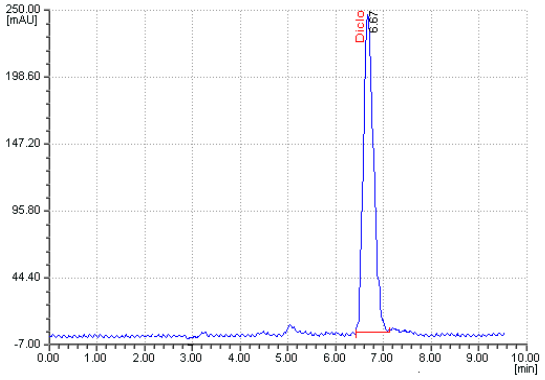


Fig. 15: Chromatogram of 50 µg/mL of Dicloxacillin

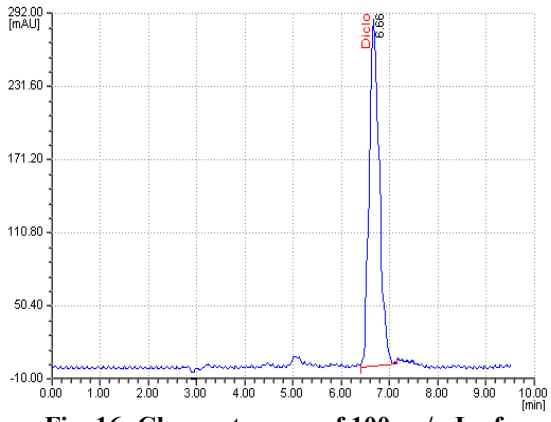


Fig. 16: Chromatogram of 100 µg/mL of Dicloxacillin

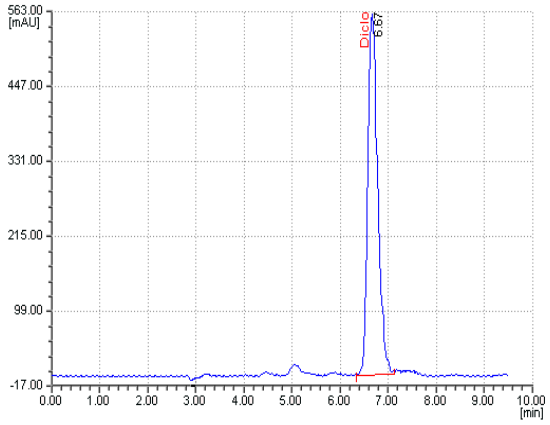


Fig. 17: Calibration curve of Dicloxacillin

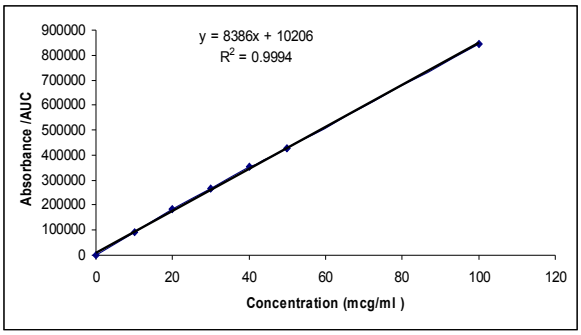


Table. 5 : Calibration of Cefixime and Dicloxacillin

S.No.	Conc. (µg/mL)		Area (µV. sec)	
	Cefixime	Dicloxacillin	Cefixime	Dicloxacillin
1	10	10	94574.0	92845.7
2	20	20	192020.4	181902.1
3	30	30	291273.8	268036.1
4	40	40	378547.9	354648.6
5	50	50	475633.9	426203.4
6	100	100	945467.8	844311.8

C. Selection of mobile system: The pure drug of Cefixime and Dicloxacillin were injected into HPLC system by using different solvent systems. Different mobile phases like aetonitrile: methanol: water, acetonitrile: methanol: potassium dihydrogen phosphate buffer, methanol: potassium dihydrogen phosphate buffer, were tried in order to find the optimum conditions for the seperation of Cefixime and Dicloxacillin. Finally the optimum composition of the mobile phase was selected as methanol (75mL) mixed with 0.05M phosphate buffer (25mL), whose pH was adjusted to 4.5 by ortho- phosphoric acid which gave satisfactory results with sharp well defined and well resolved peaks and acceptable peak parameters as compared to other mobile phases.

D. Preparation of mobile phase: Methanol (75mL) was mixed with 0.05M phosphate buffer (25 mL), and then pH was adjusted to 4.5 by addition of ortho-phosphoric acid.

E. Degassing the mobile phase: The mobile phase prepared was degassed by ultrasonication for about 20 min, so as to avoid the disturbances caused by dissolved gases.

F. Filtration of mobile phase: The mobile phase after degassing was filtered through 0.45 µm membrane nylon filter to remove the smaller particles that may be present in the mobile phase & which may cause clogging of column.

G. Preparation of standard stock solutions: 10 mg of Cefixime and 10 mg of Dicloxacillin were weighed seperately and dissolved in 50ml of mobile phase with shaking. Then it was sonicated and the volume was made upto 100 mL by using mobile phase. From standard stock solution of drug, appropriate dilutions were made using the mobile phase & the sample was filtered through 0.2 µm of clear filtrate were injected into the HPLC column.

H. Loading of mobile phase: Filtered & degassed mobile phase was loaded in the reservoir. Priming was done for each freshly prepared mobile phase.

I. Baseline stabilization: The detector was turned on for an hour before the actual run in order to obtain the stable UV light. The mobile phase run was started at desired flow rate and the run was continued until the stable baseline was obtained.

J. Loading of samples: Well prepared & filtered samples of Cefixime and Dicloxacillin were loaded into the Rheodyne injector port using a syringe and the sample was then injected.

K. Washing the column: Once the analysis of samples was finished, the column was first washed by flushing with the mobile phase for half an hour, afterwards with double distilled water & methanol in 1:1 proportion for another one hour.

L. Selection & Optimization of HPLC method: After the selection of suitable mobile phase, it was then optimized for its reproducibility, sensitivity and accuracy.

M. Chromatogram of working standard for Cefixime and Dicloxacillin

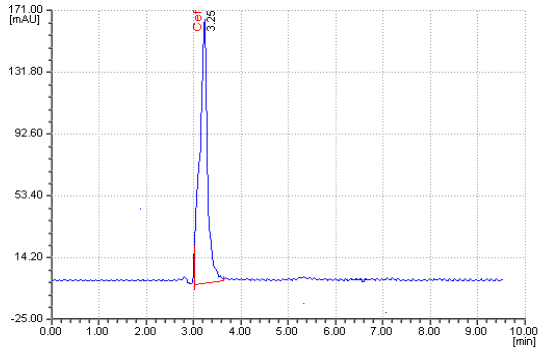


Fig. 18: Chromatogram of working standard of 20 µg/ml of cefixime

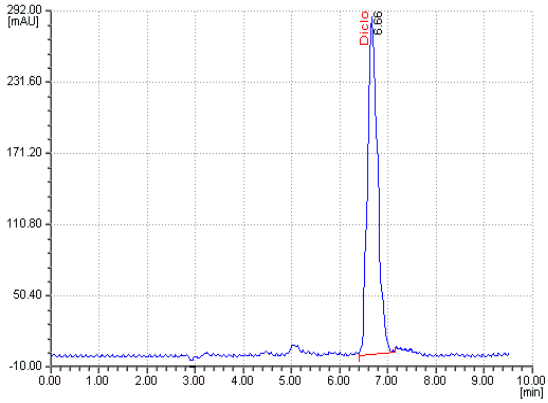


Fig. 19: Chromatogram of working standard 50µg/ml of Dicloxacillin

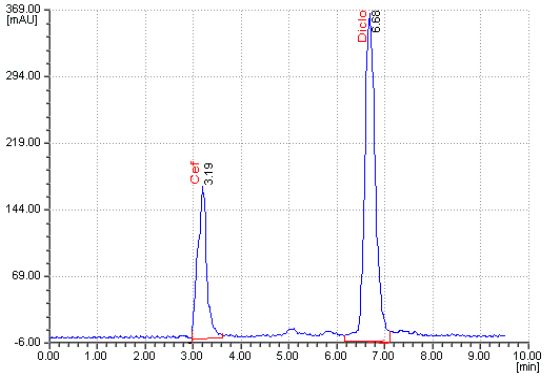


Fig. 20: Chromatogram of working standard of 20µg/ml of Cefixime and 50µg/ml of Dicloxacillin

Table. 6 : Details of chromatogram of working standard containing Cefixime and Dicloxacillin.

Sr. No	Name	RT (min)	Area (µV.sec)	Theoritical plates	Resolution	Asymmetry
1	Cefixime	3.194	184282.7	2450.41	2.51	1.45
2	Dicloxacillin	6.683	417470.4	3470.89	2.42	1.26

N. Analysis of Tablet formulation:

Brand: HIFEN-LXX 200

Each Tablet contains:

Cefixime 200mg

Dicloxacillin 500 mg

Procedure: Take twenty tablets, each containing 200 mg of Cefixime and Dicloxacillin 500 mg .The tablets were crushed to fine powder and amount of powder equivalent 100 mg of Cefixime and 250 mg Dicloxacillin were weighed and transferred to100 mL dried volumetric flask. Sufficient amount of mobile phase was added to dissolve the content and shaken for 20 min. The volume was made up to 100 mL with mobile phase. Then solution was filtered by using membrane filter and digassed. From this solution appropriate dilutions of Cefixime and Dicloxacillin were made to get the final concentrations and injected into the system to get the chromatogram. The chromatogram obtained is shown in fig no.20 and the area obtained in each chromatogram of five replicate was correlated with regression equation and the amount found is calculated which was within the limit of label claim as mentioned in table no.11.

Fig. 21: Chromatogram of Cefixime (20 µg/mL) & Dicloxacillin (50 µg/mL tablet) in Tablet Formulation.

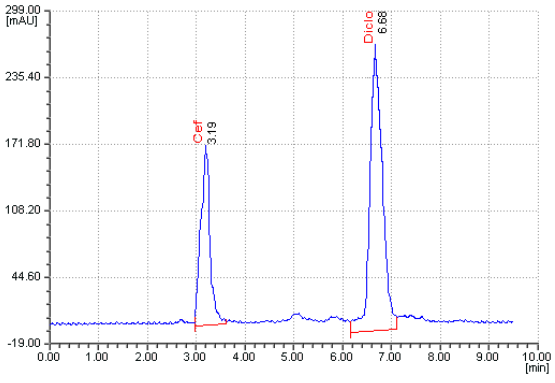


Table. 7: Details of chromatogram of Cefixime and Dicloxacillin.

Sr. No	Name	RT (min)	Area (µV sec)	Therotical plates	Resolution	Asymmetry
1	Cefixime	3.191	191454.5	2173.41	2.64	1.39
2	Dicloxacillin	6.684	423811.4	3430.89	2.42	1.93

Table 8: Analysis of HIFEN-LXX 200 Tablet.

Sr. no.	Amount present in(µg)		Amount found in(µg)		% Label claim	
	Cefixime	Dicloxacillin	Cefixime	Dicloxacillin	Cefixime	Diclo
1	20	50	19.94	49.75	99.73	99.50
2	20	50	19.94	49.67	99.71	99.35
3	20	50	19.96	49.60	99.81	99.20
4	20	50	19.91	49.73	99.59	99.47
5	20	50	19.89	49.86	99.47	99.73

O. Evaluation of analytical method (Method validation):

❖ Linearity and sensitivity:

Suitable dilutions of different concentrations using mobile phase were made from the standard stock solutions. The linearity of the relationship between peak area and concentration was determined by analyzing six working standards over the concentration range 10-100 µg/ml for Cefixime and 10-100 µg/ml for Dicloxacillin. Here the peak areas were recorded against concentration & graph of concentration Vs peak area was plotted. Results obtained are shown in table below.

Table. 9 : Linearity of Cefixime. (n=3)

Standard conc.→	10 µg/mL	20 µg/mL	30 µg/mL	40 µg/mL	50 µg/mL	100 µg/mL
Replicates	Peak area					
1	94574	192020.4	291273.8	378547.9	475633.9	945467.8
2	93966.1	192256.1	291611.4	378810.1	475841.2	945211.7
3	94914.6	192411.7	291426.4	378947.4	476022.1	945569.4
Mean	94484.9	192229.4	291437.2	378768.5	475832.4	945416.3
± SD	480.48	197.01	169.05	202.97	194.24	184.32
%RSD	0.5085	0.1024	0.0580	0.0535	0.040	0.0194

Fig. 22: Calibration plot of Cefixime

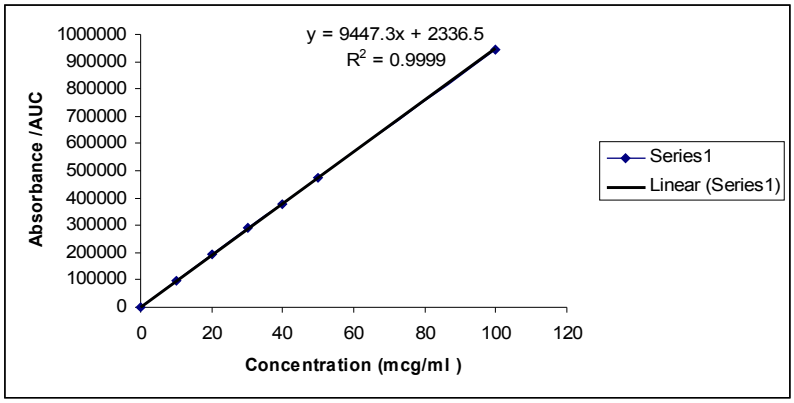
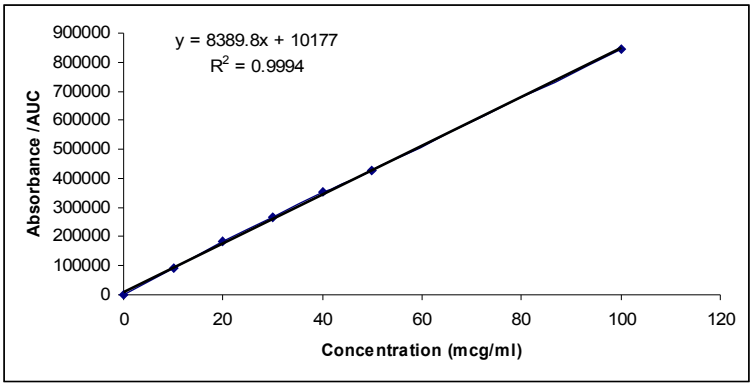


Table.10: Linearity Dicloxacillin. (n=3)

Standard conc.→	10µg/mL	20µg/mL	30µg/mL	40µg/mL	50µg/mL	100µg/mL
Replicates	Peak area					
1	92845.7	181902.1	268036.1	354648.6	426203.4	844311.8
2	92805.1	181714.4	268214.3	354821.1	426501.4	844621.1
3	92817.4	181874.2	268411.8	354919.1	426412.6	844974.9
Mean	92822.7	181830.2	268220.7	354796.3	426372.5	844635.9
± SD	20.818	101.27	187.93	136.94	153.0	331.79
% RSD	0.0224	0.0556	0.070	0.0385	0.035	0.039

Fig. 23: Calibration of Dicloxacillin



Precision: Repeatability of method was established by analyzing various replicates standards of Cefixime and Dicloxacillin. All the solutions were analyzed thrice, in order to record any intra-day & inter-day variation in the result. The result obtained for intraday variations are shown in table no.11, 12. & the result obtained for inter-day variations are shown in the table no.13, 14.

Table. 11: Intra-day variability of Cefixime

Conc. (µg/mL)	Peak area (µV. sec)			Mean area (µV sec)	± SD	%RSD
	Trial 1	Trial 2	Trial 3			
20	192020.4	191841.2	191928.4	191930.0	89.61	0.0466
30	291273.8	291411.7	291574.6	291420.0	150.57	0.051
40	378547.9	378634.2	378859.1	378680.4	160.66	0.0424

Table. 12: Intra-day variability of Dicloxacillin

Conc. (µg/mL)	Peak area (µV. sec)			Mean area (µV sec)	± SD	% RSD
	Trial 1	Trial 2	Trial 3			
20	181902.1	181711.2	181633.4	181748.9	138.26	0.076
30	268036.1	268224.2	268474.5	268244.9	219.93	0.081
40	354648.6	354801.7	354919.7	354790.0	135.92	0.0383

Table. 13: Inter-day variability of Cefixime

Conc. (µg/mL)	Peak area (µV. sec)			Mean area (µV. sec)	± SD	%RSD
	Day 1	Day 2	Day 3			
20	192414.4	192636.6	192811.4	192620.8	198.97	0.1032
30	291414.8	291605.1	291547.1	291522.3	97.53	0.0334
40	378878.1	378901.4	378793.4	378857.6	56.83	0.0150

Table. 14: Inter-day variability of Dicloxacillin.

Conc. (µg/mL)	Peak area (µV. sec)			Mean area (µV sec)	± SD	%RSD
	Day 1	Day 2	Day 3			
20	182011.4	182244.8	182334.1	182196.8	166.62	0.0914
30	268211.5	268347.5	268474.1	268344.4	131.32	0.0489
40	354648.6	354841.4	354939.2	354809.7	147.86	0.0416

Accuracy: To check the accuracy of proposed method, level of recovery carried out at 80, 100 and 120% of the concentration as per standard addition method. Here the product was accessed by addition of series of known amount of standard drug in the product and then the contents were estimated by assay method .The % recovery for Cefixime & Dicloxacillin is in the range. Results are shown in table no.15.

Table.15: Recovery studies of Cefixime and Dicloxacillin.

Tablet sample	Level of recovery %	Amount taken (µg/mL)		Amount of standard added (µg/ml)		Total amount recovered (µg/mL)		%Recovery	
	Drug	CEF	DICLO	CEF	DICLO	CEF	DICLO	CEF	DICLO
	80	20	50	16	40	35.90	89.89	99.72	99.87
	80	20	50	16	40	35.87	89.99	99.63	99.98
	80	20	50	16	40	35.91	89.94	99.75	99.93
	100	20	50	20	50	39.97	99.64	99.92	99.64
	100	20	50	20	50	39.96	99.72	99.90	99.72
	100	20	50	20	50	39.93	99.58	99.82	99.58
	120	20	50	24	60	43.98	109.76	99.95	99.78
	120	20	50	24	60	43.94	109.91	99.86	99.91
	120	20	50	24	60	43.96	109.84	99.90	99.85

Table. 16: Statistical Validation of HIFEN-LXX 200 Tablet

Level of % recover	% Mean		± SD		Standard error of mean	
	Cefixime	Dicloxacillin	Cefixime	Dicloxacillin	Cefixime	Dicloxacillin
80	99.70	99.92	0.0624	0.0550	0.0625	0.0551
100	99.88	99.64	0.0529	0.0702	0.530	0.0704
120	99.91	99.84	0.0450	0.0650	0.0452	0.0651

Limit of detection (LOD): LOD is calculated from the formula

LOD = 3.3σ/S

σ= Standard deviation of the response, s = slope of the calibration curve

Cefixime: 0.0831383 µg/mL

Dicloxacillin: 0.0610809 µg/mL

Limit of Quantitation (LOQ): LOQ is calculated from the formula

LOQ=10σ/S

σ= Standard deviation of the response S= slope of the calibration curve

Cefixime: 0.2519344 µg/mL

Dicloxacillin: 0.1850938 µg/mL

Linearity Range: The range shown by is given as follows

Cefixime: 10-100 µg/mL

Dicloxacillin: 10-100 µg/mL

Robustness: Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase ratio and pH was altered. Variation of mobile phase pH and ratio are seemed to have greater impact on resolution and hence it should be meticulously controlled.

Analysis of tablet formulation:

After analysis of all the different brands of tablets the amount found is calculated which was within the limit of label claim.

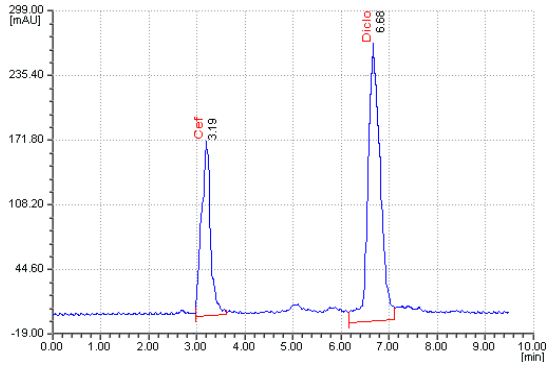


Fig. 24: Chromatogram of Cefixime (20 µg/mL) & Dicloxacillin (50 µg/mL tablet) in Tablet Formulation.

Table. 17: Analysis of tablet

S. No.	Amount present in (µg)		Amount found in (µg)		% Label claim	
	Cef.	Diclo.	Cef.	Diclo.	Cef.	Diclo.
1	20	50	19.94	49.75	99.73	99.50
2	20	50	19.94	49.67	99.71	99.35
3	20	50	19.96	49.60	99.81	99.20
4	20	50	19.91	49.73	99.59	99.47
5	20	50	19.89	49.86	99.47	99.73

3. Result and Discussion

3.1 HPLC Method:

RP-HPLC method was developed and validated for estimation of Cefixime and Dicloxacillin from bulk drug and tablet formulation. Method was validated by using different parameters likes linearity, precision, accuracy, limit of detection, limit of quantitation, specificity, range and robustness.

Table 18: Optimized parameters for HPLC method

Parameters	Chromatographic conditions
HPLC System	Cyberlab- Chrom-HPLC. V4.0
Pump	LC-P-100
Detector	LC- UV 100 (UV Visible)
Column	Capcell Pack C-18 (4.6mm x 250 mm)
Column temperature	Ambient
Mobile phase	Methanol: phosphate buffer. (75:25).
Concentration of standard solution	100 µg/mL of Cefixime and 100 µg/mL of Dicloxacillin in mobile phase.
Detection of Wavelength	232 nm
Flow rate	1mL/min
Sample volume	10 µL
Run time	9.5 min
Retention time	Cefixime: 3.19 min, Dicloxacillin: 6.68 min

Method of RP High performance liquid chromatography has been successfully employed for simultaneous estimation of Cefixime and Dicloxacillin from bulk and tablet dosage form. Method was validated as per ICH and USP guidelines by using different parameters like linearity, precision, accuracy, limit of detection, limit of quantitation, specificity, range and robustness. The linearity of Cefixime was observed in the range of 10-100 µg/mL and that of Dicloxacillin was in the range of 10-100 µg/mL. Detection wavelength used was 232 nm with correlation coefficient 0.9999 and 0.9994 for Cefixime and Dicloxacillin respectively. The limit of detection was found to be 0.0831383 µg/mL and 0.0610809 µg/mL for Cefixime and Dicloxacillin respectively & limit of quatitation 0.2519344 µg/mL and 0.1850938 µg/mL for Cefixime and Dicloxacillin respectively. The percent relative standard deviation and high percent recovery data were satisfactory and confirms accuracy, precision and reliability of the method. Thus the methods are simple, accurate, precise, economical and reproducible for routine estimation of Cefixime and Dicloxacillin respectively from bulk drug & formulations.

4. Conclusion

Conclusion for HPLC Method: Attempts were made to develop RP-HPLC method for simultaneous estimation of Cefixime and Dicloxacillin from tablet formulation. RP-HPLC method was developd and validated as per ICH and USP guidelines by using Methanol: 0.05M phosphate buffer, (75:25 v/v) pH 4.5 as mobile phase, Retention time of Cefixime and Dicloxacillin were found to be 3.19 min and 6.68 min respectively. The wavelength used was 232 nm and flow rate was 1.0 mL/min. The method was found to be simple, accurate, precise, economical and reproducible. So the propose method can be used for the routine quality control analysis of Cefixime and Dicloxacillin in bulk drug as well as tablet formulations.

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