International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (4): 216-225
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Pharmaceutical Sciences | Open Access | MCI Approved

A New RP-HPLC Method for The Simultaneous Estimation of Cefpodoxime and Clavulanic Acid in It's Pure and Pharmaceutical Dosage Form as Per ICH Guidelines

Sravani V and Srinivas A*
Department of Pharmaceutical Analysis, Unity College of Pharmacy, Raigir, Bhongir, Yadadri bhuvanagiri, Telangana, India.

Received: 02 Jul 2019 / Accepted: 04 Aug 2019 / Published online: 1 Oct 2019
***Corresponding Author Email:** drampaty@gmail.com

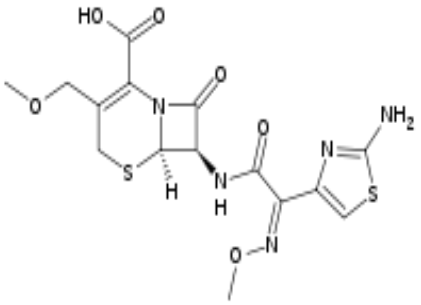
Abstract
A simple, specific, precise and accurate Stability indicating RP-HPLC method for simultaneous estimation of Cefpodoxime and Clavulanic Acid in its pure and pharmaceutical dosage form has been developed and validated as per ICH Guidelines. The separation was achieved by Phenomenex Gemini ODS C18 (4.6mm×250mm) 5µm column and Acetonitrile: Methanol: Water (55:25:20% v/v) used as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 229 nm. Retention time of Cefpodoxime and Clavulanic Acid were found to be 2.157 min and 3.631 min respectively. The method has been validated for linearity, accuracy, precision, robustness, LOD and LOQ. Linearity observed for Cefpodoxime 10 – 30µg/ml and for Clavulanic Acid 6 - 14µg/ml. Developed method was found to be accurate, precise and simple, specific for simultaneous estimation of Cefpodoxime and Clavulanic Acid in pure form and their Combined Pharmaceutical Dosage Form. The precision results are not more than 2%. The proposed method was successfully applied for the simultaneous estimation of both the drugs in commercial combined dosage form.

Keywords
Cefpodoxime and Clavulanic Acid, RP-HPLC, Validation, ICH Guidelines.

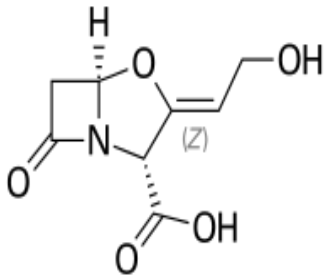
*****

INTRODUCTION
Cefpodoxime proxetil and Clavulanic acid are antibacterial drugs. Cefexime is chemically: (6R, 7R)-7-{[2-(2-amino-1,3-thiazol-4-yl)-2 carboxy methoxy imino) acetyl] amino}-3 (methoxy methyl)-8-oxo-5-thia- 1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid. It is a III generation Cephalosporin antibiotic that acts by inhibiting cell wall synthesis. It has a molecular weight of 427.455 gMo/l. Cefpodoxime is soluble in organic solvents such as ethanol, methanol, DMSO and Acetonitrile. Cefpodoxime proxetil is a prodrug that is absorbed from the gastrointestinal tract. Cefpodoxime is active against a wide spectrum of Gram-positive and Gram-negative bacteria. Cefpodoxime is stable in the presence of beta-lactamase enzymes. As a result, many organism's

resistant to penicillin's and cephalosporin's, due to their production of beta-lactamase, may be susceptible to cefpodoxime. Clavulanic acid is (2R, 5R, Z)-3-(2-hydroxyethylidene)- 7-oxo-4-oxa-1-aza-bicyclo [3.2.0] heptane-2-carboxylic acid. Freely soluble in water, slightly soluble in ethanol (96 per cent), very slightly soluble in acetone. Clavulanic acid competitively and irreversibly inhibits a wide variety of beta-lactamases, commonly found in microorganism's resistant to penicillin's and cephalosporin's. Binding and irreversibly inhibiting the beta-lactamase results in a restauration of the antimicrobial activity of beta-lactam antibiotics against lactamase-secreting-resistant bacteria. By inactivating beta-lactamase (the bacterial resistance protein), the accompanying penicillin /cephalosporin drugs may be made more potent as well. Literature survey revealed that several methods were reported for cefpodoxime proxetil and clavulanic acid individually and in combinations (S.Low et al, 1989, S.Thomas et al, 2010, Raj K 2010, Prabhu et al 2010, Aghazadeh A and Kazemifard G 2001, B.Thomas et al, 2010, Krzysztof P, Owski and Tyski S 2001, Kim D 2009, Malathi et al 2010, M. Foulstone and C. Reading, 1982, Nanda RK, Gaikwad Prakash A 2009 and Neto A et al, 2009). Therefore, the main objective of this study was to attempt to develop a simple and rapid analytical method for simultaneous estimation of cefixime trihydrate and clavulanate potassium in a single dosage form and validate the proposed assay.



Cefedoxime



Clavulanic acid

MATERIALS AND METHODS

Materials: Analytical pure samples of Cef (% purity - 99.98) and Pot. clav. (% purity -98.70) (Emcure Pharmaceutical, India) were used in the study. The pharmaceutical dosage form used in this study was Cepodem XP 325 tablet (Ranbaxy Pharmaceutical, India) procured from local market and labeled to contain 200 mg of Cef and 125 mg of Pot. clav. per tablet. The solvents and chemicals used in the study were of analytical-grade (Qualigens fine chemicals, Mumbai).

Instrumentation: Waters Alliance 2695 HPLC with PDA Detector 996 model.

Preparation of mobile phase:

Accurately measured 750ml of Acetonitrile (75%) of and 250ml of HPLC Water (25%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Cefpodoxime and Clavulanic Acid working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette out 0.2ml of Cefpodoxime and 0.1ml of Clavulanic Acid from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution:

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Cefpodoxime and Clavulanic Acid sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Filter the sample solution by using injection filter which contains 0.45µ pore size.

Further pipette out 0.2ml of Cefpodoxime and 0.1ml of Clavulanic Acid from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water and ACN: Water with varying proportions. Finally, the mobile phase was optimized to Acetonitrile and water in proportion 75:25 v/v respectively.

Optimization of Column:

The method was performed with various C18 columns like Symmetry, X terra and ODS column. Phenomenex Gemini C18 (4.6×250mm) 5µ was found

to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used : Waters Alliance 2695 HPLC with PDA Detector 996 model.
Temperature: 36ºC
Column: Phenomenex Gemini ODS C18 (4.6mm× 250mm) 5µm
Mobile phase : Acetonitrile: Methanol: Water (55:25:20% v/v)
Flow rate: 1ml/min
Wavelength: 229nm
Injection volume :10µl
Run time: 6minutes

METHOD VALIDATION

PREPARATION OF MOBILE PHASE:

Preparation of mobile phase:

Accurately measured 750ml of Acetonitrile (75%) of and 250ml of HPLC Water (25%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

VALIDATION PARAMETERS

SYSTEM SUITABILITY

Accurately weigh and transfer 10 mg of Cefpodoxime and Clavulanic Acid working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette out 0.2ml of Cefpodoxime and 0.1ml of Clavulanic Acid from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

SPECIFICITY STUDY OF DRUG:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Cefpodoxime and Clavulanic Acid working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette out 0.2ml of Cefpodoxime and 0.1ml of Clavulanic Acid from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution:

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Cefpodoxime and Clavulanic Acid sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Filter the sample solution by using injection filter which contains 0.45µ pore size.

Further pipette out 0.2ml of Cefpodoxime and 0.1ml of Clavulanic Acid from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure:

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

PREPARATION OF DRUG SOLUTIONS FOR LINEARITY:

Accurately weigh and transfer 10 mg of Cefpodoxime and Clavulanic Acid working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Preparation of Level – I (10ppm of Cefpodoxime and 6ppm of Clavulanic Acid):

Pipette out 0.1ml of Cefpodoxime and 0.06ml of Clavulanic Acid in to a 10ml volumetric flask and make the volume up to mark by using diluent and sonicate for air entrapment.

Preparation of Level – II (15ppm of Cefpodoxime and 8ppm of Clavulanic Acid):

Pipette out 0.15ml of Cefpodoxime and 0.08ml of Clavulanic Acid in to a 10ml volumetric flask and make the volume up to mark by using diluent and sonicate for air entrapment.

Preparation of Level – III (20ppm of Cefpodoxime and 10ppm of Clavulanic Acid):

Pipette out 0.2ml of Cefpodoxime and 0.1ml of Clavulanic Acid in to a 10ml volumetric flask and

make the volume up to mark by using diluent and sonicate for air entrapment.

Preparation of Level – IV (25ppm of Cefpodoxime and 12ppm of Clavulanic Acid):

Pipette out 0.25ml of Cefpodoxime and 0.12ml of Clavulanic Acid in to a 10ml volumetric flask and make the volume up to mark by using diluent and sonicate for air entrapment.

Preparation of Level – V (30ppm of Cefpodoxime and 14ppm of Clavulanic Acid):

Pipette out 0.3ml of Cefpodoxime and 0.14ml of Clavulanic Acid in to a 10ml volumetric flask and make the volume up to mark by using diluent and sonicate for air entrapment.

Procedure:

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

PRECISION

REPEATABILITY

Preparation of Cefpodoxime and Clavulanic Acid Product Solution for Precision:

Accurately weigh and transfer 10 mg of Cefpodoxime and Clavulanic Acid working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette out 0.2ml of Cefpodoxime and 0.1ml of Clavulanic Acid from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

INTERMEDIATE PRECISION:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

DAY 1:

The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.

DAY 2:

The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.

Accuracy:

For preparation of 50% Standard stock solution:

Accurately weigh and transfer 10mg of Cefpodoxime and Clavulanic Acid working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette out 0.1ml of Cefpodoxime and 0.05ml of Clavulanic Acid from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 100% Standard stock solution:

Accurately weigh and transfer 10 mg of Cefpodoxime and Clavulanic Acid working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette out 0.2ml of Cefpodoxime and 0.1ml of Clavulanic Acid from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 150% Standard stock solution:

Accurately weigh and transfer 10 mg of Cefpodoxime and Clavulanic Acid working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette out 0.3ml of Cefpodoxime and 0.15ml of Clavulanic Acid from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure:

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Cefpodoxime and Clavulanic Acid and calculate the individual recovery and mean recovery values.

ROBUSTNESS:

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

For preparation of Standard solution:

Accurately weigh and transfer 10 mg of Cefpodoxime and Clavulanic Acid working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette out 0.2ml of Cefpodoxime and 0.1ml of Clavulanic Acid from the above stock solutions into

a 10ml volumetric flask and dilute up to the mark with Diluent.

Effect of Variation of flow conditions:

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of mobile phase organic composition:

The sample was analyzed by variation of mobile phase i.e. Acetonitrile: Methanol and water was taken in the ratio and 50:30:20, 60:20:20 instead of 55:25:20 remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase ratio : Acetonitrile: Methanol: Water (55:25:20% v/v)
Column : Phenomenex Gemini ODS C18 (4.6mm×250mm) 5µm
Column temperature : 36ºC
Wavelength : 229nm
Flow rate : 1ml/min
Injection volume : 10µl
Run time : 6minutes

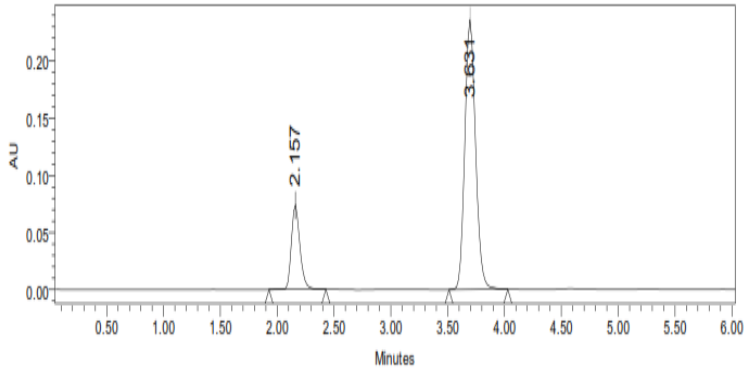


Figure-: Optimized Chromatogram (Standard)

Table 1-: Optimized Chromatogram (Standard)

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Cefpodoxime	2.157	62354	7548	1.9	8564	
2	Clavulanic Acid	3.631	128568	85698	1.8	9542	8.64

Observation: From the above chromatogram it was observed that the Cefpodoxime and Clavulanic Acid peaks are well separated and they show proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

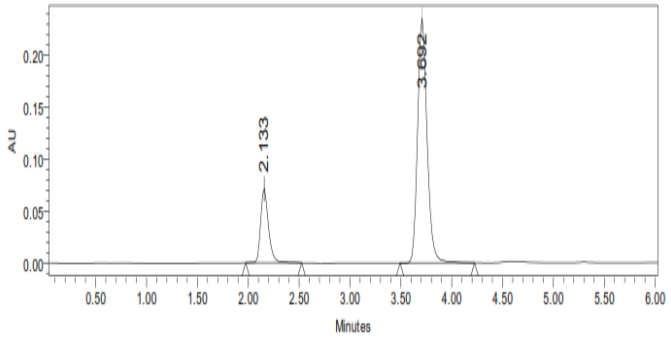


Figure-: Optimized Chromatogram (Sample)

Table 2-: Optimized Chromatogram (Sample)

S.No.	Name	Rt	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Cefpodoxime	2.133	63564	7685	1.10	8695	
2	Clavulanic Acid	3.692	129865	86598	1.90	9658	9.25

Acceptance criteria:

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

VALIDATION

Table 3-: Results of system suitability for Cefpodoxime

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Cefpodoxime	2.152	62356	7568	8569	1.9
2	Cefpodoxime	2.157	62584	7522	8575	1.9
3	Cefpodoxime	2.141	62365	7586	8536	1.8
4	Cefpodoxime	2.133	62587	7548	8594	1.8
5	Cefpodoxime	2.166	62658	7542	8514	1.9
Mean			62510			
Std. Dev.			139.6872			
% RSD			0.223464			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

Table 4-: Results of system suitability for Clavulanic Acid

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)			Resolution
1	Clavulanic Acid	3.674	128585	85462	9568	1.8	9.56
2	Clavulanic Acid	3.631	128698	85745	9578	1.9	9.56
3	Clavulanic Acid	3.625	128754	85475	9587	1.8	9.57
4	Clavulanic Acid	3.692	128457	85687	9568	1.9	9.56
5	Clavulanic Acid	3.629	128754	85745	9536	1.8	9.56
Mean			128649.6	85674	9514	1.9	9.57
Std. Dev.			127.8761				
% RSD			0.099399				

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitate Cefpodoxime and Clavulanic Acid in drug product.

Table 5-: Peak results for assay standard of Cefpodoxime

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Cefpodoxime	2.152	62356	7584	1.9	8578	1
2	Cefpodoxime	2.198	62548	7684	1.8	8594	2
3	Cefpodoxime	2.179	62854	7569	1.9	8569	3

Table 6-: Peak results for assay standard of Clavulanic Acid

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Clavulanic Acid	3.646	12856	8856	1.8	9586	1
2	Clavulanic Acid	3.604	12875	8547	1.9	9587	2
3	Clavulanic Acid	3.610	12868	5856	1.9	9575	3

Table 7-: Peak results for Assay sample of Cefpodoxime

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Cefpodoxime	2.152	63586	7698	1.20	8695	1
2	Cefpodoxime	2.150	63587	7659	1.20	8698	2
3	Cefpodoxime	2.187	63658	7648	1.21	8652	3

Table 8-: Peak results for Assay sample of Clavulanic Acid

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Clavulanic Acid	3.646	129865	86598	1.9	9658	1
2	Clavulanic Acid	3.651	129786	86574	1.9	9628	2
3	Clavulanic Acid	3.601	129785	86598	1.8	9687	3

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY OF CEFPODOXIME AND CLAVULANIC ACID:

Table 9:

Concentration µg/ml	Average Peak Area
10	35479
15	52598
20	68654
25	84816
30	102548

Table 10:

Concentration µg/ml	Average Peak Area
6	85987
8	102587
10	128569
12	135847
14	146859

REPEATABILITY

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table 11-: Results of Repeatability for Cefpodoxime:

S. No.	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Cefpodoxime	2.157	62355	7586	8569	1.9
2	Cefpodoxime	2.159	62354	7584	8542	1.9
3	Cefpodoxime	2.186	62357	7524	8574	1.9
4	Cefpodoxime	2.160	62358	7534	8534	1.8
5	Cefpodoxime	2.170	62359	7598	8568	1.8
Mean			62356.6			
Std.dev			2.073644			
%RSD			0.003325			

Acceptance Criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table 12-: Results of Repeatability for Clavulanic Acid:

S. No.	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Clavulanic Acid	3.603	128568	8568	9542	1.8
2	Clavulanic Acid	3.608	128564	8547	9546	1.7
3	Clavulanic Acid	3.600	128475	8598	9578	1.8
4	Clavulanic Acid	3.696	128564	8547	9528	1.8
5	Clavulanic Acid	3.629	128754	8564	9575	1.7
Mean			128585			
Std.dev			102.2644			
%RSD			0.079531			

ACCURACY:

Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated.

Table 12-: Results of Accuracy for concentration-50%

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Cefpodoxime	2.165	34868	3778	0.98	4658	1
2	Clavulanic Acid	3.696	64578	64528	1.01	5284	1
3	Cefpodoxime	2.155	34827	3789	0.99	4755	2
4	Clavulanic Acid	3.684	64624	64528	1.02	5369	2
5	Cefpodoxime	2.173	34859	3796	0.98	4856	3
6	Clavulanic Acid	3.688	64586	64854	1.01	5364	3

Table 13-: Results of Accuracy for concentration-100%

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Cefpodoxime	2.156	68958	7659	1.8	8598	1
2	Clavulanic Acid	3.618	127569	86598	1.8	9659	1
3	Cefpodoxime	2.226	68872	7669	1.9	8695	2
4	Clavulanic Acid	3.650	127854	86597	1.7	9625	2
5	Cefpodoxime	2.226	68943	7625	1.9	8624	3
6	Clavulanic Acid	3.650	127586	86958	1.7	9685	3

Table 14-: The accuracy results for Cefpodoxime

% Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	34851.33	10.034	10	100.340	
100%	68924.33	20.079	20	100.395	100.348%
150%	102889	30.093	30	100.310	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

Table 15-: The accuracy results for Clavulanic Acid

% Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	64596	5.041	5	100.820	
100%	127586	10.011	10	100.110	100.492%
150%	191854	15.082	15	100.546	

- The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Table 16-: Results for Robustness

CEFPODOXIME

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	62354	2.157	8564	1.9
Less Flow rate of 0.9 mL/min	65658	2.210	8154	1.58
More Flow rate of 1.1 mL/min	61245	2.184	8264	1.69
Less organic phase	60448	2.200	8415	1.78
More Organic phase	63698	2.172	8365	1.79

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CLAVULANIC ACID

Table 17:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	128568	3.631	9542	1.8
Less Flow rate of 0.9 mL/min	134515	4.498	9254	1.7
More Flow rate of 1.1 mL/min	126854	3.505	9126	1.6
Less organic phase	124512	4.504	9245	1.4
More organic phase	122564	3.512	4954	1.6

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Cefpodoxime and Clavulanic Acid bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps. Cefpodoxime is soluble in organic solvents such as ethanol, methanol, DMSO, and Acetonitrile. It is poorly soluble in water. Clavulanic Acid is freely soluble in water, slightly soluble in ethanol (96 per cent), very slightly soluble in acetone. Acetonitrile: Methanol: Water (55:25:20% v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Cefpodoxime and Clavulanic Acid in bulk drug and in Pharmaceutical dosage forms.

REFERENCES

1. Aghazadeh A and Kazemifard G (2001). Determination of amoxycillin and clavulanic acid in pharmaceutical dosage forms by HPLC with amperometric detection. J. Sci. I. R. Iran.12: 127-131.
2. B. Thomas, S. B. Dighe, R. K. Nandaa, L. P. Kothapalli, S. N. Jagdale & A. D. Deshpande NV (2010) A validated stability indicating HPTLC method for simultaneous estimation of cefpodoxime proxetil and potassium Clavulanate in bulk and tablet dosage p. 1689-1703
3. Kim D, King J, Zuccarelli L and Ferris C (2009). Clavulanic acid: A competitive inhibitor of beta lactamase with novel anxiolytic like activity and minimal side effect. Pharmacol Biochem Behav 2: 112-120.
4. Krzysztof P, Owski and Tyski S (2001). Capillary electrophoresis versus LC for simultaneous determination of amoxicillin/ clavulanic acid and ampicillin / sulbactam in pharmaceutical formulation for injection. Int J Chem Tech Res 2: 918-923.
5. M. Foulstone and C. Reading, “Assay of Amoxicillin and Clavulanic Acid, the Component of Augmentin, in Biological Fluids with High Performance Liquid Chromatography,” Antimicrobial Agents and Chemotherapy, Vol. 22, No. 5, November 1982, pp. 753-762
6. Malathi S, Dubey R and Venkatnarayanan (2009). Simultaneous RP-HPLC estimation of cefpodoxime proxetil and clavulanic acid in tablets. Int J Pharma Rec Res 2: 45-48.
7. Nanda RK, Gaikwad Prakash A (2009). Simultaneous spectrophotometric estimation of cefixime and ornidazole in tablet dosage form. Int.J. PharmTech Res 1(3): 488-491.
8. Neto A, Hirata D, Cassiano Filho L and Bandino A (2005). A study on clavulanic acid production by streptomyces clavuligerus in batch, fed-batch and continuous processes. Brazilian J Chem Eng 22: 557-563
9. Prabhu S, Amirtharaj R Vijay, Senthilkumar. Simultaneous RP-HPLC method development and

validation of cefixime and ofloxacin in tablet dosage form.,2010, page-367-369.
10. Raj K, Yada D, Prabu C and Manikantan S (2010). Determination of cefixime trihydrate and cefuroxime axetil in bulk drug and pharmaceutical dosage forms by HPLC. Int J Chem Tech Res 2: 334-336.
11. S. Low, R. B. Taylor and J. M. Gould, "Determination of Clavulanic Acid by a Sensitive HPLC Method," Journal of Antimicrobial Chemotherapy, Vol. 24, No. (Suppl)B, 1989, pp. 83-86
12. Sharma B, Instrumental methods of chemical analysis,19th edition, Goel Publishing House, 2003.
13. S. Sharif, I. U. Khan, M. Ashfaq, M. S. Iqbal, S. Ahmad Development and validation of a high performance liquid chromatographic method for the simultaneous determination of potassium clavulanate and cefadroxil in synthetically prepared tablets. October 2010, Volume 65, pp 1029-1034