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RP-HPLC Method Development and Validation of Determination of Cefadroxil and Potassium Clavulanate Tablets

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Abstract:

Cefadroxil (CEF) and Potassium Clavulanate (CLV) are used in combination as an antibacterial tablet formulation for the treatment of various bacterial infections including respiratory tract, skin, and urinary tract infections. A simple, sensitive, rapid, precise and accurate reverse phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for the simultaneous determination of Cefadroxil and Potassium Clavulanate in combined tablet dosage form. Chromatographic separation was achieved on an *Agilent C18 (250 × 4.6 mm, 5 µm)* column using a mobile phase consisting of Potassium dihydrogen phosphate buffer: Acetonitrile: Methanol in the ratio of **20:70:10 v/v** adjusted to pH 5.0 with Orthophosphoric acid, at a flow rate of 1.0 mL/min. UV detection was performed at 225 nm with an injection volume of 20 µL. The retention time for Potassium Clavulanate and Cefadroxil was found to be **1.98 min and 6.08 min** respectively. The method was validated as per ICH Q2(R1) guidelines for parameters including system suitability, specificity, linearity, precision, accuracy, ruggedness, and robustness. The linearity was established in the concentration range of 25–125 µg/mL for Clavulanate and 100–500 µg/mL for Cefadroxil with a correlation coefficient (r^2) of 0.999 for both drugs. The developed method is simple, economical, and reproducible and can be employed for routine quality control analysis of the combined tablet formulation.

Keywords: Cefadroxil, Potassium Clavulanate, RP-HPLC, ICH validation, Simultaneous estimation, Pharmaceutical formulation.

Introduction:

Cefadroxil is a first-generation cephalosporin antibiotic that inhibits bacterial cell wall synthesis by binding to specific Penicillin-binding proteins (PBPs). It is used to treat infections caused by susceptible organisms including skin, throat (tonsillitis), urinary tract, and reproductive tract infections. Its molecular formula is C16H17N3O5S with a molecular weight of 363.39 g/mol.

Potassium Clavulanate is a β-lactamase inhibitor that forms a covalent bond with the serine residue in the active site of β-lactamase enzymes, irreversibly inhibiting them. It broadens the antibacterial spectrum of Cefadroxil against resistant organisms. Its molecular formula is C8H8NO5K with a molecular weight of 237.25 g/mol.

The combination of Cefadroxil and Potassium Clavulanate is commercially

available as Cefadrox®-CV tablets. A thorough literature survey revealed that limited RP-HPLC methods have been reported for the simultaneous estimation of both drugs in their combined dosage form. Most of the available methods involve tedious sample preparation, longer run times, or are validated for single-drug estimation only. The present work focuses on developing a simple, rapid, and cost-effective RP-HPLC method for the simultaneous determination of Cefadroxil and Potassium Clavulanate in tablet dosage form and validating it as per ICH Q2(R1) guidelines.

Solutions of Cefadroxil and Potassium Clavulanate were scanned separately and as a combined solution over the UV range of 200–400 nm. Both compounds showed satisfactory absorbance at 225 nm, which was selected as the detection wavelength for the simultaneous estimation of both drugs by RP-HPLC.

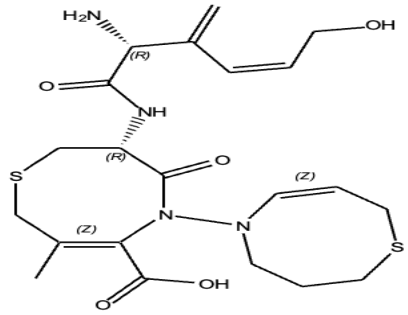


Figure 1a: Chemical structure of Cefadroxil

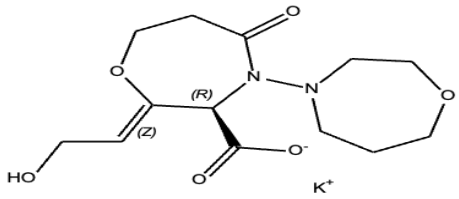


Figure 1b: Chemical structure of Potassium Clavulanate

Materials and Methods:

Preparation of Mobile Phase

6.8 g of Potassium dihydrogen phosphate was dissolved in 1000 mL of Milli-Q water and the pH was adjusted to 5.0 ± 0.1 with dilute Orthophosphoric acid. This buffer was mixed with HPLC grade Acetonitrile and Methanol in the ratio of 20:70:10 v/v. The prepared mobile phase was filtered through a 0.45 µm Nylon membrane filter and degassed by sonication for 5 minutes before use.

Preparation of Standard Stock and Working Solutions

Cefadroxil: 102.5 mg was accurately weighed, transferred into a 100 mL volumetric flask, dissolved, and diluted to volume with the diluent (mobile phase). Potassium Clavulanate: 29.1 mg was accurately weighed, transferred into a 100 mL volumetric flask, dissolved, and diluted to volume with the diluent. Mixed standard solution was prepared by taking 25 mL each from both stock solutions into a 50 mL volumetric flask and making up to volume with the diluent. This gave a working concentration of approximately 250 µg/mL for Cefadroxil and 62.5 µg/mL for Clavulanate.

Method Development and Optimization

The optimized chromatographic conditions were finalized after systematic evaluation of column type, mobile phase composition, pH, flow rate, and detection wavelength to achieve adequate resolution (≥ 2.0), good peak symmetry, and a short run time. The optimized conditions are presented in Table 1a.

Table 1a: Optimized Chromatographic Conditions for the Proposed RP-HPLC Method

Parameter	Chromatographic Conditions
Column	Agilent C18 (250 × 4.6 mm, 5 µm)
Mobile Phase	Potassium dihydrogen phosphate buffer: Acetonitrile: Methanol (20:70:10 v/v), pH 5.0
Flow Rate	1.0 mL/min
Detection Wavelength	225 nm
Injection Volume	20 µL
Column Temperature	25°C (Ambient)
Run Time	10 minutes
Detector	UV Detector
Diluent	Mobile phase

Method Validation

The method was validated as per ICH Q2(R1) guidelines for the following parameters: system suitability, specificity, linearity, system precision, method precision, accuracy, ruggedness, and robustness.

System Suitability

System suitability was assessed by injecting six replicate injections of the mixed standard solution into the HPLC system. Parameters including retention time, peak area response, and %RSD were calculated. The results are presented in Table 1b.

Table 1b: System Suitability Parameters

stem Suitability Parameter	Limits	Cefadroxil	Clavulanate	Result
Retention Time (min)	—	6.085	1.980	—
Tailing Factor (T)	≤ 2.0	—	—	Complies
Theoretical Plates	NLT 2000	4254	—	Complies
% RSD (6 injections)	NMT 2.0	0.8207	0.3330	Complies

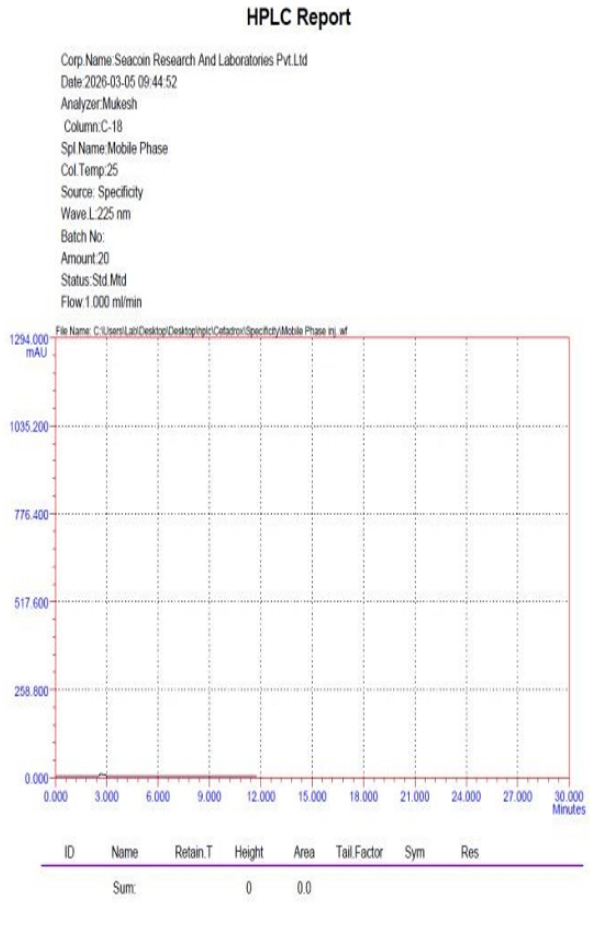
* Average of six determinations. SD = Standard deviation. RSD = Relative standard deviation.

Specificity

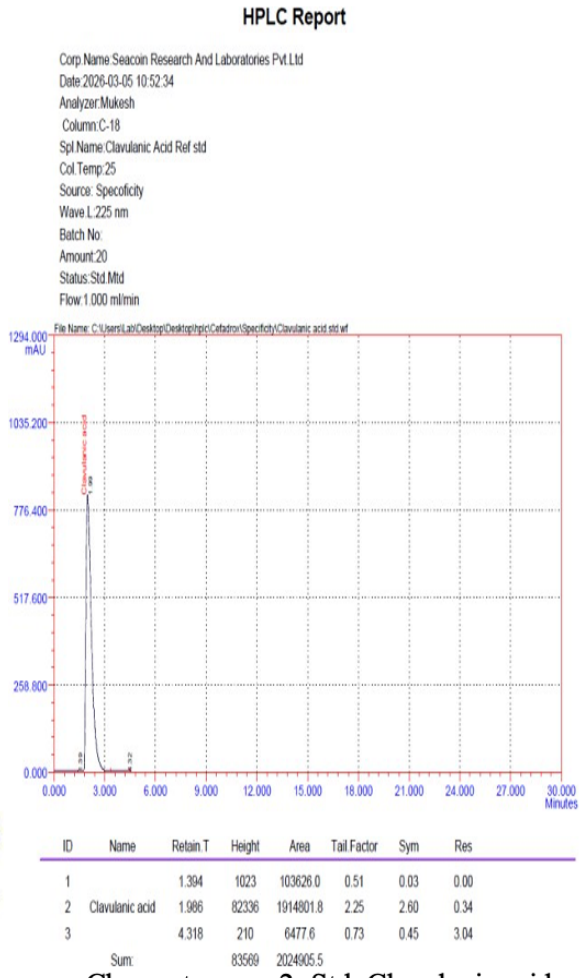
Blank (diluent) and mobile phase injections showed no peaks at the retention times of Clavulanate (1.98 min) and Cefadroxil (6.085 min), confirming that the solvents and excipients used in the formulation do not interfere with the estimation. The results are presented in Table 2.

Table 2: Results of Specificity Study for Cefadroxil and Potassium Clavulanate

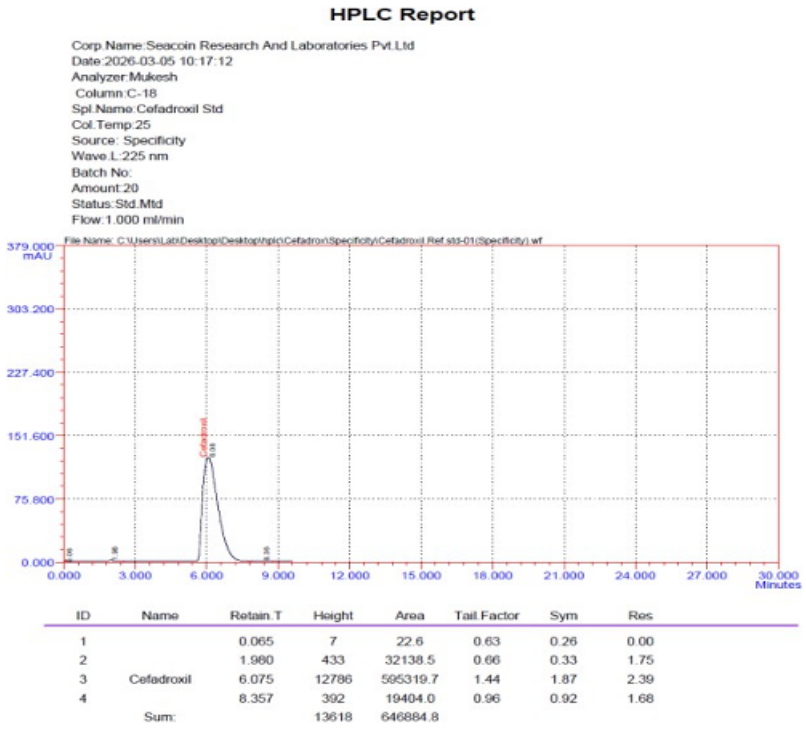
Solution	ntion Time (min)	Peak Purity	Inference
Diluent	—	—	No interference at RT of analyte peaks
Mobile Phase (Blank)	—	—	No interference at RT of analyte peaks
Clavulanate Standard	1.980	—	No interference
Clavulanate Sample	1.980	Pass	No interference
Cefadroxil Standard	6.085	—	No interference
Cefadroxil Sample	6.085	Pass	No interference



Chromatogram 1- Mobile Phase



Chromatogram 2- Std. Clavulanic acid



Chromatogram 3- Std. Cefadroxil

Precision

System Precision: Six replicate injections of the mixed standard solution were injected into the HPLC system. The values of %RSD for peak area responses are given in Table 2a.

Table 2a: System Precision Data

Inj. No.	**adroxil RT (min)**	**Cefadroxil Area**	**ulanate RT (min)**	**avulanate Area**
1	6.09	292473	1.98	778874
2	6.09	297435	1.98	786292
3	6.09	293817	1.98	783367
4	6.09	290153	1.98	784541
5	6.09	294610	1.98	786237
6	6.09	291042	1.98	785997
Mean		**293255**		**784218**
SD		2406.781		2611.818
% RSD		**0.8207**		**0.3330**
Acceptance Criteria: % RSD should be NMT 2.0%.				

Linearity

The linearity of both drugs was determined over the following concentration ranges: Clavulanate: 25–125 µg/mL and Cefadroxil: 100–500 µg/mL. Five concentrations were prepared from stock solutions and injected into the HPLC system. The peak area responses were recorded and calibration curves were plotted. The linearity data are presented in Tables 2b and 2c.

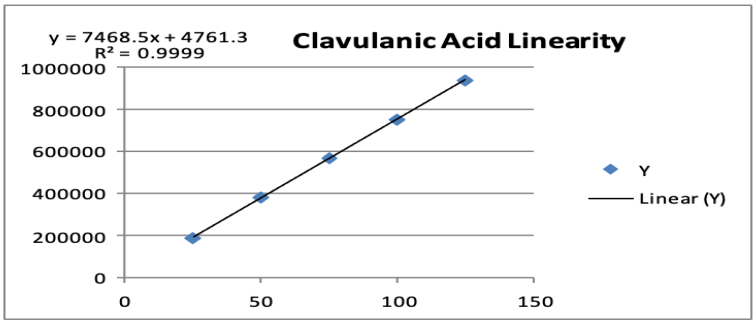
Table 2b: Linearity Data of Potassium Clavulanate

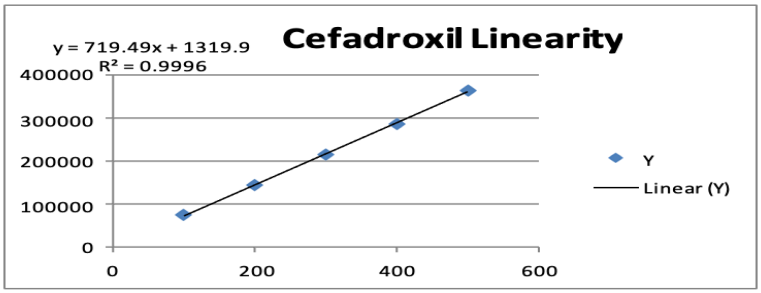
Conc. (µg/mL)	Area Response	Avg. Area Response
25	187716, 187754, 187471	187647
50	380646, 381175, 380938	380919
75	568745, 569684, 569099	569176
100	748357, 749269, 752873	750166
125	939080, 935661, 935019	936586
Regression Equation		**y = 7468x + 4761**
Correlation Coefficient (r^2)		**0.999**

Table 2c: Linearity Data of Cefadroxil

Conc. (µg/mL)	Area Response	Avg. Area Response
100	74538, 75472, 75210	75073
200	144230, 140262, 149048	144513
300	216575, 213107, 213939	214540
400	289855, 286151, 282118	286041
500	363911, 357131, 361060	360700
Regression Equation		**y = 719x + 1319**
Correlation Coefficient (r^2)		**0.999**

Acceptance Criteria: Correlation coefficient (r^2) should be NLT 0.999.





Accuracy (Recovery Studies)

Accuracy was determined by spiking known amounts of both drugs into placebo at three concentration levels (80%, 100%, 120% of the test concentration) in triplicate. The % recovery was calculated and the results are presented in Tables 3a and 3b.

Table 3a: Results of Accuracy Study

Level	Drug	Amount Added (mg)	Amount Recovered (mg)	% Recovery	% RSD
80%	Cefadroxil	400.0	398.5	99.6	0.28
80%	Clavulanate	100.0	99.3	99.3	0.35
100%	Cefadroxil	500.0	500.87	100.17	0.19
100%	Clavulanate	125.0	125.09	100.07	0.22
120%	Cefadroxil	600.0	599.1	99.85	0.31
120%	Clavulanate	150.0	149.8	99.87	0.26

Acceptance Criteria: % Recovery should be within 98.0%–102.0%.

Robustness

The robustness of the method was evaluated by deliberately varying method parameters such as flow rate (±0.1 mL/min), detection wavelength (±5 nm), pH of mobile phase (±0.2 units), organic phase ratio (±2%), and column temperature (±5°C). The system suitability parameters remained within acceptable limits under all tested conditions, demonstrating the robustness of the method. Results are summarized in Table 3b.

Table 3b: Results of Robustness Study

Parameter	Optimized	ariation Used	% RSD (Area)	Remarks
Flow rate (mL/min)	1.0	0.9	1.12	*Robust
Flow rate (mL/min)	1.0	1.0	0.82	Robust
Flow rate (mL/min)	1.0	1.1	1.05	*Robust
Wavelength (nm)	225	220	1.23	Robust
Wavelength (nm)	225	225	0.83	Robust
Wavelength (nm)	225	230	1.41	Robust
pH of Mobile Phase	5.0	4.8	1.18	*Robust
pH of Mobile Phase	5.0	5.0	0.82	Robust
pH of Mobile Phase	5.0	5.2	1.30	*Robust

Acceptance criteria: % RSD NMT 2.0. * Significant change in Retention time.

Ruggedness

Ruggedness was assessed by performing the assay on two different HPLC instruments (LC-CYBER-LAB and SHIMADZU System-1) using two different columns (Agilent C18 and Phenomenex C8) by different analysts on different days. The % RSD for assay values in all cases was found to be within 2.0%, confirming the ruggedness of the developed method.

Results and Discussion

A simple and sensitive RP-HPLC method was developed and validated for the simultaneous estimation of Cefadroxil and Potassium Clavulanate in combined tablet dosage form. Multiple mobile phase compositions were evaluated before finalizing Potassium dihydrogen phosphate buffer: Acetonitrile: Methanol (20:70:10 v/v) at pH 5.0 as the optimal mobile phase. This composition provided well-resolved, symmetrical chromatographic peaks for both analytes within a run time of 10 minutes.

The retention times of Potassium Clavulanate and Cefadroxil were found to be 1.98 min and

6.085 min, respectively. The number of theoretical plates (4254) indicated efficient column performance. The UV detection wavelength of 225 nm was selected as both drugs showed adequate absorbance at this wavelength.

The linearity of both analytes was demonstrated over the concentration ranges of 25–125 µg/mL (Clavulanate) and 100–500 µg/mL (Cefadroxil), with correlation coefficients (r^2) of 0.999 for both, confirming excellent linearity. The %RSD for system precision was 0.8207% and 0.3330% for Cefadroxil and Clavulanate respectively, well within the NMT 2.0% limit. The % recovery in accuracy studies ranged from 99.3% to 100.17% for both drugs, within the acceptable range of 98.0%–102.0%. The ruggedness and robustness studies confirmed that the method is reliable under variable analytical conditions.

Application of the Developed Method for Marketed Formulation (Assay)

For the assay, 20 tablets of Cefadrox®-CV (Cefadroxil 500 mg + Potassium Clavulanate 125 mg) were weighed and the average weight was calculated. A quantity of powder equivalent to

102.5 mg of Cefadroxil and 29.1 mg of Clavulanate was accurately weighed, transferred into 100 mL volumetric flasks, sonicated for 30 minutes, filtered through 0.45 µm nylon membrane filters, and diluted appropriately before HPLC injection. The assay results are presented in Table 4a.

Table 4a: Assay Results of Marketed Formulation (Cefadrox®-CV)

Drug	Label Claim (mg/tab)	Amount Found (mg/tab)	% Labeled Amount	Mean % Recovery ± SD	% RSD
Cefadroxil	500	500.87	100.174%	100.17 ± 0.8	0.80
Potassium Clavulanate	125	125.09	100.07%	100.07 ± 0.6	0.60

* Average of two determinations. SD denotes standard deviation. RSD denotes % relative standard deviation.

Summary

The methods employed and results obtained in the study are summarized below in Table 4b.

Table 4b: Summary of RP-HPLC Method Validation for Cefadroxil and Potassium Clavulanate

S.No.	Validation Parameter	Acceptance Criteria	Results
1	System Suitability	1. %RSD of six replicate injections should be NMT 2.0%. 2. Theoretical plates NLT 2000. 3. Tailing factor ≤ 2.0.	Cefadroxil %RSD: 0.8207 Clavulanate %RSD: 0.3330 Plates: 4254 – Complies
2	Specificity	No interference from blank, diluent, mobile phase, or excipients at retention times of analytes.	No interference observed at RT of Cefadroxil (6.085 min) and Clavulanate (1.98 min). Complies.
3	Precision (System)	% RSD for six replicate injections should be NMT 2.0%.	Cefadroxil: % RSD – 0.8207 Clavulanate: % RSD – 0.3330
4	Linearity	Correlation coefficient (r^2) should be NLT 0.999.	Cefadroxil: r^2 = 0.999 Clavulanate: r^2 = 0.999
5	Accuracy Study	Mean % recovery should be between 98.0% to 102.0%.	Cefadroxil: 99.6%–100.17% Clavulanate: 99.3%–100.07% Complies
6	Ruggedness	% RSD for assay across different instruments/analysts should be NMT 2.0%.	Within limits. Complies.
7	Robustness	% RSD NMT 2.0% upon deliberate variation of method parameters (flow rate, wavelength, pH).	Within limits under all tested conditions. Complies.

Conclusion

A simple, rapid, precise, accurate, and reproducible RP-HPLC method has been developed and validated for the simultaneous determination of Cefadroxil and Potassium Clavulanate in combined tablet dosage form as per ICH Q2(R1) guidelines. The method employs an Agilent C18 (250 × 4.6 mm, 5 µm) column with a mobile phase of Potassium dihydrogen phosphate buffer: Acetonitrile: Methanol (20:70:10 v/v), pH 5.0, at a flow rate of 1.0 mL/min, with UV detection at 225 nm.The chromatographic peaks of Potassium Clavulanate (RT: 1.98 min) and Cefadroxil (RT: 6.085 min) were well separated within a run time of 10 minutes. The method was found to be linear (r^2= 0.999 for both drugs), precise (%RSD < 1%), accurate (% recovery 99.3%–100.17%), and robust. The assay of marketed Cefadrox®-CV tablets gave % labeled amounts of 100.174% for Cefadroxil and 100.07% for Potassium Clavulanate, confirming the applicability of the method for routine quality control analysis.The RP-HPLC method offers advantages of low solvent consumption, short run time, good peak symmetry, and simultaneous

estimation of both drugs in a single chromatographic run. Hence, it can be recommended for routine pharmaceutical quality control analysis of Cefadroxil and Potassium Clavulanate combined tablet formulations.

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