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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF CEFADROXIL AND CLAVULANIC ACID IN PHARMACEUTICAL DOSAGE FORM

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Abstract: A simple and precise two UV-spectrophotometric and one RP-HPLC methods have been developed for the simultaneous estimation of Cefadroxil and Clavulanic acid in pharmaceutical dosage form. The first UV spectrophotometric method was a determination using the simultaneous equation method at 231 nm and 288 nm over the concentration range 8-40 µg/ml and 2-10 µg/ml for Cefadroxil and Clavulanic acid, respectively. The mean recoveries obtained for Cefadroxil and Clavulanic acid were in the range of 99.43-100.7 and 98.5-100%. The second UV method was a determination using the first order derivative spectroscopy at 258 nm and 264 nm over the concentration range of 8-40 and 2-10µg/ml for Cefadroxil and Clavulanic acid, respectively. The mean recoveries obtained for Cefadroxil and Clavulanic acid were in the range of 98.40-100.7 and 99.4-100.6 %. In RP-HPLC analysis is carried out using Water: Methanol: Triethylamine (85: 15: 0.1), adjusted pH-5.5 with 1% H_3PO_4as the mobile phase at a flow rate of 1ml/min and Purospher® RP-C18 (4.6 mm i.d×250 mm) column as stationery phase with detection wavelength of 285 nm. Linearity was obtained in the concentration range of 8-40 and 2-10 µg/ml for Cefadroxil and Clavulanic acid, respectively. The retention time was found to be 3.840 and 5.580 min for Cefadroxil and Clavulanic acid. The mean recoveries obtained for Cefadroxil and Clavulanic acid were in the range of 99.58-100.10 and 99.45-100.01%. The developed method has been statistically validated according to ICH guidelines and found to be simple, precise and accurate with the prescribed values. Thus the proposed method was successfully applied for the estimation of Cefadroxil and Clavulanic acid in routine quality control analysis in bulk and its formulations.

Keywords: Cefadroxil, Clavulanic acid, RP-HPLC, simultaneous equation method, first order derivative spectroscopy.

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INTRODUCTION

Cefadroxil (CEFA), chemically is (6R, 7R)-7-[(2R)-2-amino-2-(4-hydroxyphenyl) acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid. It is an Antibacterial Agents used in bacterial infections. Clavulanic acid (CLAV), chemically, is (2R, 3Z, 5R)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo [3.2.0] heptane-2-carboxylic acid. It is ß-lactamase inhibitor used in infections. CEFA is official in IP and CLAV is official in IP and BP. These two drugs are marketed as combined dose tablet formulation in the ratio of 500:125 mg (CEFA: CLAV). Literature survey revealed that a number of methods have been reported for estimation of CEFA and CLAV individually or in combination with other drugs. For this combination HPTLC and LC-MS method has been reported but no method has been reported by UV and RP-HPLC. Objective of this study is to develop a Simple, fast and precise method for simultaneous estimation of Cefadroxil and Clavulanic acid by RP-HPLC and UV spectroscopy method.

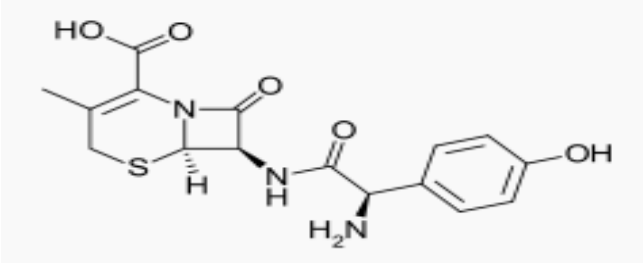


Fig1: Structure of Cefadroxil

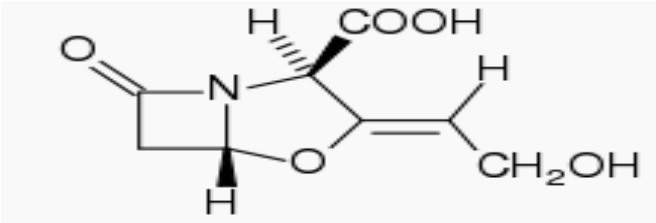


Fig2: Structure of Clavulanic acid

MATERIALS AND METHODS [3]

Instrumentation:

For UV-spectrophotometric method Double beam UV-visible spectrophotometer (Shimadzu, model 1800) having two matched quartz cells with 1 cm light path was used. For RP-HPLC method Young Lin, YL 9100 HPLC systems, YL9110 Quaternary solvent delivery Pump, YL9160 Photodiode array (PDA) detector, Purospher® RP-C18 Column (5µm) with Young Lin software for data processing was used.

MATERIALS

Standard gift sample of CEFA was received from Shantam pharmaceutical Pvt. Ltd. Ahmedabad, India, CLAV as gift sample as from Dial pharmaceutical Pvt. Ltd. Ahmedabad, India. Combined dose tablet formulation, FDC limited containing CEFA (500 mg), CLAV (125 mg) was purchased from a local pharmacy Store. Methanol used for UV-spectrophotometric method was of AR grade. TEA (Merck Chemicals, India), Water (Rankem Ltd. Ahmedabad, India) used in RP-HPLC were of HPLC grade.

Procedure

Preparation of standard stock solution

Accurately weighed 10mg CEFA and 10mg CLAV was transferred in to different two 10ml volumetric flask and dissolved in methanol and dilute upto the mark with methanol to give a stock solution having concentration of 1mg/ml (1000µg/ml). Accurately measured 1ml of above two Stock solutions was transferred in to different two 10 ml volumetric flask and diluted to the mark with methanol to obtain a working standard solution (100µg/ml) of Cefadroxil and Clavulanic acid.

Simultaneous equation method (Method A)

In simultaneous equation method (method A), for the selection of analytical wavelength , solutions of CEFA (24 µg/ml) and CLAV (6 µg/ml) were prepared separately by appropriate dilution of above standard stock solution and scanned in the spectrum mode from 200 to 400 nm. From the overlain spectra of these drugs [Figure 3], wavelengths 231 nm (λ_{max} of CEFA) and 288 nm (λ_{max} of CLAV) were selected for analysis. The calibration curves for CEFA and CLAV were prepared in the concentration range of 8-40 µg/ml and 2-10 µg/ml, respectively at the selected wavelengths. The absorbances were measured at the selected wavelengths. The molar absorptivity values were 549.2 at 231 nm and 53.9 at 288 nm for CEFA and 441.8 at 231 nm and 334.7 at 288 nm for CLAV, respectively. The absorbance and absorptivity values were substituted in the following equation to obtain the concentrations:

$Cx = A_2 ay_1 - A_1 ay_2 / ax_2 ay_1 - ax_1 ay_2$ .....(1)
$Cy = A_1 ax_2 - A_2 ax_1 / ay_1 ax_2 - ay_2 ax_1$ .....(2)

where A_1 and A_2 are absorbances of the mixture at 231 and 288 nm , respectively, ax_1 and ax_2 are absorptivity of CEFA at 231 and 288 nm , respectively, ay_1 and ay_2 are absorptivity of CLAV at 231 and 288 nm , respectively, and Cx and Cy are concentrations of CEFA and CLAV, respectively.

First order derivative method (Method B)

In first order derivative method (Method B), Solutions of 24 µg/ml and 6 µg/ml of CEFA and CLAV were prepared separately from above standard stock solution. Both the solutions were scanned in the spectrum mode from 200 to 400 nm. The absorption spectra thus obtained were derivatized from first to fourth order. First-order derivative (n=1) was selected for analysis of both the drugs. The zero crossing wavelengths 258 nm and 264 nm were selected for CEFA and CLAV, respectively [Figure 4]. The absorbances were measured at the selected wavelengths. The calibration curves for CEFA and CLAV were prepared in the concentration range of 8-40 µg/ml and 2-10 µg/ml, respectively at 258 nm and 264 nm. The concentaration are obtained from straight line equation.

Validation parameter [4]

The proposed methods were validated as per ICH guidelines.

Linearity

Linearity is expressed in terms of correlation co-efficient of linear regression analysis. The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 8-40µg/ml for Cefadroxil and 2-10µg/ml for Clavulanic acid at 231nm and 288 nm for simultaneous equation method and at zero crossing wavelengths 258 nm and 264 nm for first order derivative method. The calibration curve of absorbance vs. concentration was plotted and correlation coefficient and regression line equations for Cefadroxil and Clavulanic acid were determined. (Table 3, 6)

Precision (Repeatability)

For Repeatability, it was carried out by preparing 6 replicates of 6 same concentrations, within the linearity range and measuring the absorbance of each solution on the same day. % RSD (% relative standard deviation) was calculated. The %RSD values were found to be below 2% which indicate that the proposed methods are repeatable (Table 3, 6).

Intermediate precision (Reproducibility)

The intermediate precision for the proposed method was determined by estimating standard solution of CEFA (8, 24, 40 µg/ml) and CLAV (2, 6, 10 µg/ml) for three times on the same day (intraday) and on three different days (interday). The results are reported in terms of relative standard deviation (RSD). The RSD values were found to be below 2% which indicate that the proposed methods are reproducible (Table 3, 6)

Accuracy

The accuracy of the method was determined by calculating recoveries of CEFA and CLAV by the standard addition method. Known amount of standard of CEFA and CLAV (80%, 100%, and 120%) were added to the sample solutions of tablet dosage forms. The amounts of CEFA and CLAV were estimated by regression equation. The results are shown in (Table 9). The values prove that the method is accurate. (Table 1, 4)

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ of CEFA and CLAV were determined by using standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines. The LOD and LOQ data are presented in (Table 3, 6)

Analysis of tablet formulation

Twenty tablets were weighed accurately and powdered. A quantity of tablet Powder equivalent to 500 mg of Cefadroxil and 125mg Clavulanic acid was transferred to 100 ml volumetric Flask containing 40 ml of methanol, gently shaking was carried out for 5 min and Ultrasonicated for 5min. The volume was made up to the mark with methanol. The tablet sample solution was filtered through Whatman filter paper no. 41. 5 ml of filtrate was further diluted to 60 ml of methanol to get 5000 µg/ml concentrations. From the 5000 µg/ml of sample stock solution take 1ml of solution and diluted Up to the mark in 10ml volumetric flask to get 500µg/ml. From the 500µg/ml of sample solution take 2ml of solution and further diluted up to the mark in 10ml volumetric flask to get100µg/ml. From the 100µg/ml of sample solution, take 1.6ml of solution and further diluted up to the mark in 10ml volumetric flask to get 16µg/ml. So the final solution was made which contains 16µg/ml Cefadroxil and 4µg/ml Clavulanic acid both. In method I, the concentration of both CEFA and CLAV were determined by measuring absorbance of sample solution at 231 nm & 288 nm and using equations (1) and (2). In method II, absorbance of sample solution was measured in first order derivative mode at zero crossing wavelengths 258 nm & 264 nm. Concentration of CEFA and CLAV in the diluted solution was obtained from calibration curves. Amount of CEFA and CLAV in mg/tab was then calculated. Results of tablet analysis are shown in (Table 2, 5)

RP-HPLC (Method c)

Chromatographic conditions: Preliminary studies were conducted and trails are made for the method development. Separation and analysis was carried out on Purospher® RP-C18 column (4.6 x 250mm), 5µ particle size. The optimized mobile phase consisting of Water: methanol: Triethylamine (85:15:0.1, v/v/v), adjusted pH 5.5 with 1% $H_3PO_4$and filtered through 0.45 µm membrane filter using vacuum pump. Flow rate was maintained at 1 ml/min and run time for

10 min, prior to sample injection, column was saturated with mobile phase for 40 min and injection volume was 20 µl injected by auto sampler. The detection response was measured at 285 nm and maintained at ambient temperature.

Preparation of optimized mobile phase: Take one 500 ml volumetric flask, washed with distilled water and then methanol (AR Grade) then dried it in oven at 60˚C for 20-25 min. Methanol and Water pH-5.5 filtered through 0.45µm Chrom Tech Nylon-66 filter paper. After filteration it was sonicated for 20min on ultrasonicator. Mixing of 0.1 ml Triethylamine in 485ml Water and 15ml of Methanol (HPLC grade) pH- 5.5 adjusted with 1% H_3PO_4 in 500 ml volumetric flask.

Preparation of standard stock solution: same as UV spectroscopy method.

Validation Parameter [4]

Linearity and Range

The linearity response was determined by analyzing 5 independent levels of Calibration curve in the range of 8-40 µg/ml and 2-10 µg/ml for CEFA and CLAV Respectively. Plot the calibration curve of Area versus respective concentration and Find out correlation co-efficient and regression line equation for CEFA and CLAV. (Table 10)

Precision (Repeatability)

For Repeatability, it was carried out by preparing 6 replicates of 6 same concentrations, within the linearity range and measuring the Peak area of each solution on the same day. % RSD (% relative standard deviation) was calculated. The %RSD values were found to be below 2% which indicate that the proposed methods are repeatable (Table 10).

Intermediate precision (Reproducibility)

The intermediate precision for the proposed method was determined by estimating standard solution of CEFA (8, 24, 40 µg/ml) and CLAV (2, 6, 10 µg/ml) for three times on the same day (intraday) and on three different days (interday). The results are reported in terms of relative standard deviation (RSD). The RSD values were found to be below 2% which indicate that the proposed methods are reproducible (Table 10).

Accuracy

The accuracy of the method was determined by calculating recoveries of CEFA and CLAV by the standard addition method. Known amount of standard of CEFA and CLAV (80%, 100%, and 120%) were added to the sample solutions of tablet dosage forms. The amounts of CEFA and

CLAV were estimated by regression equation. The results are shown in (Table 7). The values prove that the method is accurate. (Table 8)

System suitability

Standard solution was injected six times into system and chromatograms were recorded, % RSD (relative standard deviation) of retention time & peak area, theoretical plates and tailing factor were calculated. (Table 7)

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ of CEFA and CLAV were determined by using standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines. The LOD and LOQ data are presented in (Table 10).

Analysis of tablet formulation

Same as UV Spectroscopy method. (Table 9)

RESULTS AND DISCUSSION

In **Simultaneous Equation method**, the linearity range was found to be 8-40µg/ml for CEFA and 2-10µg/ml for CLAV. % Assay was found to be 98.12 % and 99.62% for CEFA and CLAV respectively. Percentage recovery for CEFA was 99.43-100.7%**,** while for CLAV it was found to be in range of 98.5-100.0%. LOD and LOQ values were found to be and 0.06 & 0.21 for CEFA and 0.13 & 0.40 for CLAV.

In **First Order Derivative method**, the linearity range was found to be 8-40µg/ml for CEFA and 2-10µg/ml for CLAV. % Assay was found to be 99.61% and 99.03% for CEFA and CLAV respectively. Percentage recovery for CEFA was 98.43-100.7%**,** while for CLAV, it was found to be in range of 99.4-100.6 %. LOD and LOQ values were found to be 0.49 & 1.5 for CEFA and 0.16 & 0.5 for CLAV.

In **RP-HPLC** method was developed and validated. The mobile phase used was water: Methanol: TEA (85:15:0.1, v/v/v), adjusted pH 5.5 with 1% H_3PO_4. The retention time of CEFA and CLAV was found to be 3.840 min and 5.580 min respectively. Linearity range was found to be 8-40 µg/ml for CEFA and 2-10 µg/ml for CLAV. % Assay was found to be 98.87% and 99.44 % for CEFA and CLAV respectively. Percentage recovery for CEFA was 99.58-100.1%**,** while for CLAV, it was found to be in range of 98.45-100.0 %. LOD and LOQ values were found to be for 0.27 & 0.82 CEFA and 0.12 & 0.39 for CLAV.

For method A:

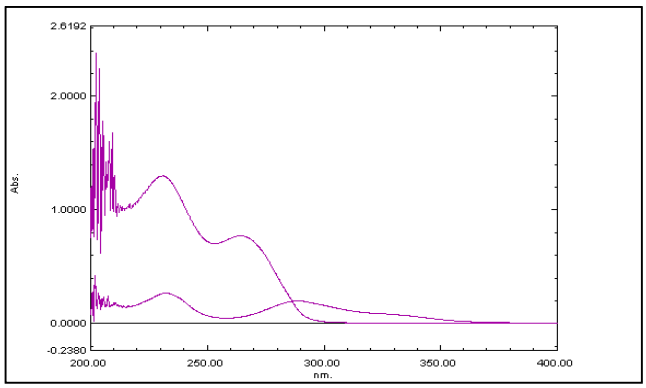


Fig3: Overlay spectra of CEFA (24µg/ml) and CLAV (6 µg/ml) for Simultaneous equation method

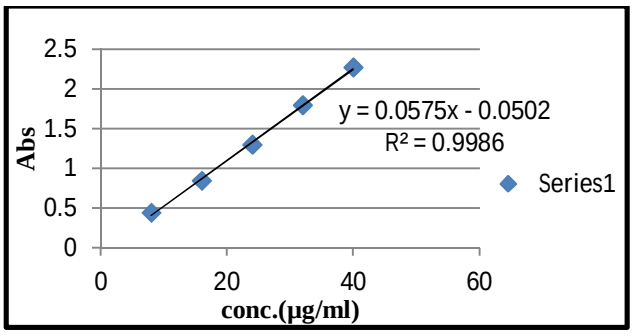
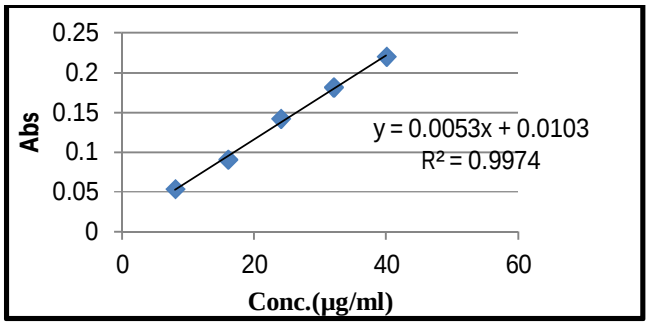


Fig4: Calibration curve of standard CEFA at 231nm



- **Fig5: Calibration curve of standard CEFA at 288nm**

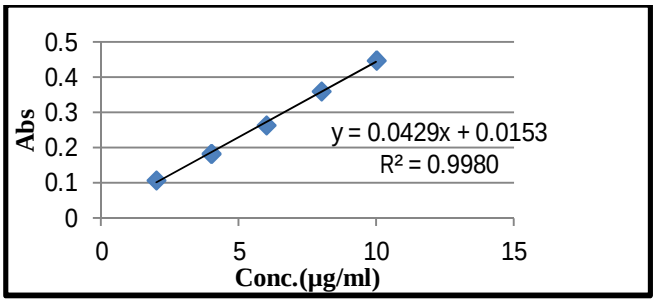


Fig6: Calibration curve of standard CLAV at 231nm

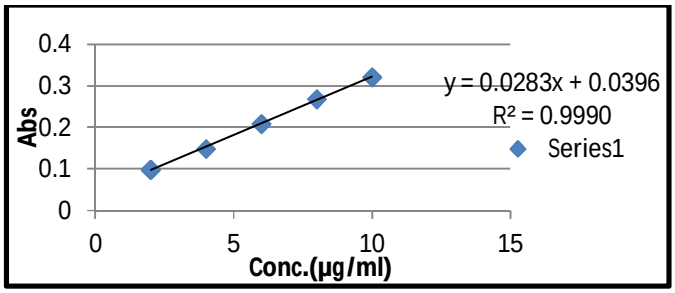


Fig7: Calibration curve of standard CLAV at 288nm

Table 1: Recovery studies data.

Drugs	% Level	Amount of drug taken (µg/ml)	Amount of std. drug added (µg/ml)	Mean amount found (µg/ml) ± S.D.	% Recovery	% RSD
CEFA	80	16	12.8	28.4 ± 0.03	100.7	0.10
	100	16	16	31.9 ± 0.04	99.84	0.12
	120	16	19.2	35 ± 0.15	99.43	0.42
CLAV	80	4	3.2	7.2 ± 0.03	100	0.41
	100	4	4	7.8 ± 0.02	98.5	0.25
	120	4	4.8	8.8 ± 0.02	100	0.22

Table 2: Assay of tablet formulation.

Tablet	Drug	Label claim (mg)	Amount found (mg) ± S.D. (n=3)	%label claim
Zadro CV	CEFA	500	490.62 ± 0.175	98.12
	CLAV	125	124.53 ± 0.370	99.62

Table 3: Summary of validation parameter of Simultaneous equation method.

Parameters	CEFA		CLAV	
	231nm	288nm	231nm	288nm
Linearity and range (µg/ml)	8-40	8-40	2-10	2-10
Accuracy (Recovery %) (n=3)	99.43 - 100.7		98.5 – 100	
Precision (%RSD)				
Intra-day (n=3)	0.06 - 0.24	0.59 - 0.93	0.43 - 0.81	0.26 - 0.92
Inter-day (n=3)	0.07 - 0.28	0.64 - 1.28	0.48 - 1.17	0.31 - 0.98
Repeatability (n=6)	0.11	0.81	0.63	0.62
LOD (µg/ml)	0.06		0.13	
LOQ (µg/ml)	0.21		0.40	
Assay % (n=3)	98.12		99.62	

For method B:

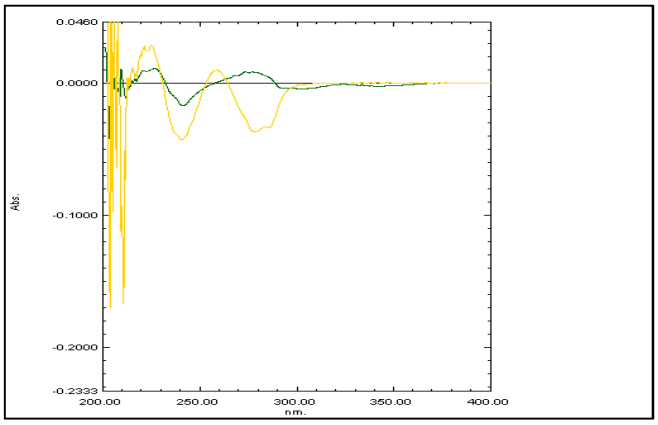


Fig 8: Overlain UV absorption spectrum of CEFA and CLAV (24and 6µg/ml) for First derivative method.

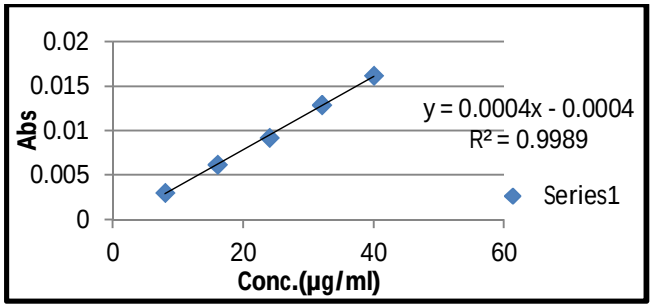


Fig 9: Calibration curve of standard CEFA at 258nm

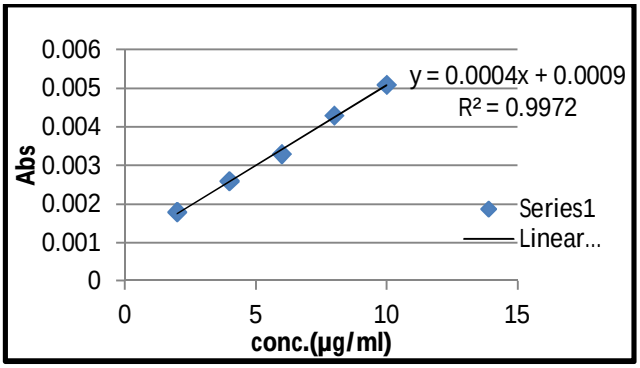


Fig 10: Calibration curve of standard CLAV at 264nm

Table 4: Recovery studies data.

Drugs	% Level	Amount of drug taken (µg/ml)	Amount of std. drug added (µg/ml)	Mean amount found (µg/ml) ± S.D.	% Recovery	% RSD
CEFA	80	16	12.8	27.7 ± 0.04	98.40	0.14
	100	16	16	32.2 ± 0.05	100.7	0.15
	120	16	19.2	35 ± 0.15	99.43	0.42
CLAV	80	4	3.2	7.25 ± 0.02	100.6	0.27
	100	4	4	7.9 ± 0.03	99.6	0.37
	120	4	4.8	8.7 ± 0.01	99.4	0.18

Table 5: Assay of tablet formulation.

Tablet	Formulation	Drug	Label claim (mg)	Amount found (mg) ± S.D.	% Amount found
Zadro CV	Tablet	Cefadroxil	500	498.07 ± 0.205	99.61
		Clavulanic acid	125	123.79 ± 0.300	99.03

Table 6: Summary of validation parameter of First order derivative method.

Parameters	CEFA	CLAV
	258nm	264nm
Linearity and Range (µg/ml)	8 – 40	2 – 10
Accuracy (Recovery %) (n=3)	98.40- 100.7	99.4-100.6
Precision (%RSD)		
Intra-day (n=3)	0.54-0.71	0.52-0.88
Inter-day (n=3)	0.76-1.23	0.92-1.50
Repeatability (n=6)	0.78	0.85
LOD (µg/ml)	0.49	1.5
LOQ (µg/ml)	0.16	0.5
Assay % (n=3)	99.61	99.03

For method C:

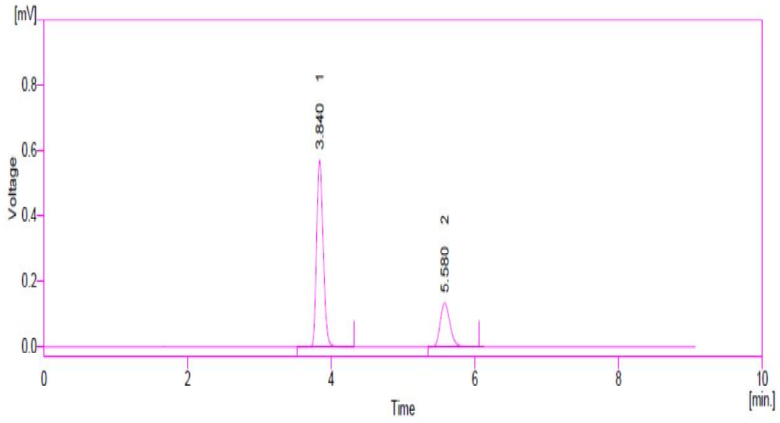


Fig 11: HPLC chromatogram.

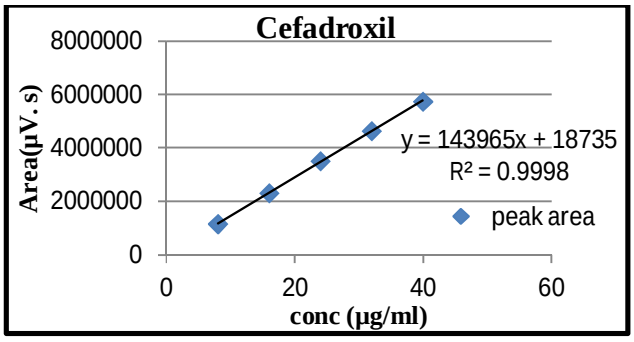


Fig 12: Calibration curve of standard CEFA

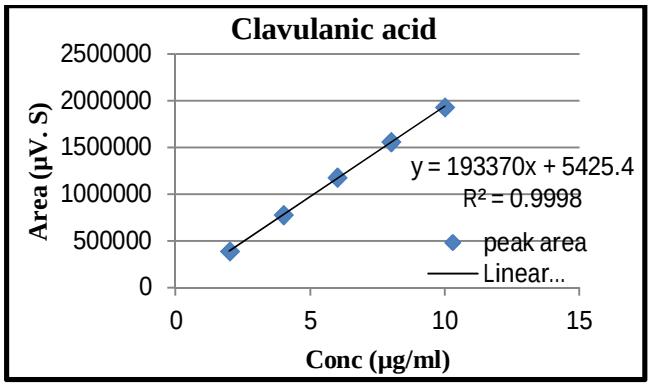


Fig 13: Calibration curve of standard CLAV

Table 7: System suitability parameters of RP-HPLC method.

Sr. no.	System suitability Parameter	Observed value Specification		Specification
		CEFA	CLAV	
1	Retention time (Rt)	3.840	5.580	
2	Resolution (Rs)	8.415		> 1.5
3	Theoretical plates(N)	8169	8396	> 2000
4	Asymmetric factor (A_f)	1.292	1.324	Not greater than 2.0

Table 8: Recovery studies data.

Drugs	% Level	Amount of drug taken (µg/ml)	Amount of std. drug added (µg/ml)	Mean amount found (µg/ml) ± S.D. (n=3)	% Recovery	% RSD
CEFA	80	16	12.8	28.00 ± 1.095	99.58	0.57
	100	16	16	31.50 ± 0.163	99.78	0.51
	120	16	19.2	35.26 ± 0.155	100.10	0.25

CLAV	80	4	3.2	7.18 ± 0.106	99.82	1.39
	100	4	4	8.00 ± 0.070	100.01	0.87
	120	4	4.8	8.66 ± 0.040	99.45	0.45

Table 9: Assay of tablet formulation.

Tablet	Formulation	Drug	Label claim (mg)	Amount found (mg) ± S.D.	% Amount found
Zadro CV	Tablet	Cefadroxil	500	494.3 ± 0.280	98.87
		Clavulanic acid	125	124.3 ± 0.400	99.44

Table 10: Summary of validation parameter of RP-HPLC method.

Parameters	CEFA	CLAV
Linearity and Range (µg/ml)	8 – 40	2 – 10
Accuracy (Recovery %) (n=3)	99.58-100.10	99.45-100.01
Precision (%RSD)		
Intra-day (n=3)	0.53-0.91	0.81-1.15
Inter-day (n=3)	0.40-0.51	0.78-1.21
Repeatability (n=6)	0.40	0.72
LOD (µg/ml)	0.27	0.12
LOQ (µg/ml)	0.82	0.39
Robustness	Robust	Robust
Assay % (n=3)	98.87	99.44

LOD = Limit of detction

LOQ = Limit of quantitation

R.S.D = Relative standard deviation

n = Number of determination

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