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THE ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF IMIPENEM & CILASTATIN BY USING RP- HPLC

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Abstract:
A new method was recognized for simultaneous estimation of Imipenem and cilastatin by RP-HPLC method. The chromatographic circumstances were effectively developed for the separation of Imipenem and cilastatin by using SYMMETRYC18 column (4.6×150mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: phosphate buffer($KH_2PO_4$and K_2HPO_4) pH 3 (pH was adjusted with orthophosphoric acid),detection wavelength was 258nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times was found to be 3.345 mins and 2.523 mins. The percentage purity of Imipenem and cilastatin was initiate to be 100.27% and 99.87% correspondingly. The system suitability parameters for Imipenem and cilastatin such as theoretical plates and tailing factor was found to be 2885, 1.25 and 2235 and 1.33, the resolution were found to be3.48. The analytical technique was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Imipenem and cilastatin were found in concentration range of 50µg-250µg and 5µg-50µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery were found to be 99.56% and 99.48%, %RSD for repeatability was 1.2 and 2, % RSD for intermediate precision was 1.1 and 1.1 respectively. The precision study was precise, robust, and repeatable.LOD value was 0.03 and 2.17,and LOQ value was 0.11 and 6.6 respectively.

Keywords: *Imipenem , cilastatin , precise, robust, and repeatable.*

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INTRODUCTION:

Chromatography is a physical method of separation in which the component to be separated are distributed between two phases of which in stationary while other moves in a definite direction (IUPAC). It deals with mainly Qualitative Method and Quantitative Method[1].Imipenem is an white to light yellow coloured powder which was soluble in water .It was store at room temperature and which was used to treat of bacterial infections (gram-positive and gram-negative microbes).Cilastatin is an white coloured powder which was soluble in water (25 mg/mL), DMSO (10 mg/mL), and methanol. It was store at room temperature and which was used as antibacterial effect. Literature review reveals that there is no analytical method reported for the analysis of Imipenem and cilastatin by simultaneous estimation by RP-HPLC[1]. Spectrophotometer, HPLC and HPTLCare the reported analytical methods for compounds either individually or in combination with other dosage form. Hence, it was felt that, there is a need of new analytical method development for the simultaneous estimation of Imipenem and cilastatin in pharmaceutical dosage form.

Present work is aimed to develop a new, simple, fast, rapid, accurate, efficient and reproducible RP-HPLC method for the simultaneous analysis of Imipenem and cilastatin. The developed technique determination be validated conferring to ICH guidelines.

MATERIALS AND METHODS:

Method development for the simultaneous estimation of Imipenem and Cilastatin by using RP-HPLC[2].

	Trail -1	Trail 2	Trail 3	Trail 4	Trail 5
Mobile phase	Methanol: H2O (50:50%v/v)	ACN: H2O (50:50%v/v)	ACN: pH 6.8 buffer (50:50 % v/v)	ACN: pH 3 buffer (65:35% v/v)	ACN: pH 3 buffer (70: 30 % v/v)
Diluent	Mobile phase was used as the diluent	Mobile phase was used as the diluent	Mobile phase was used as the diluent	Mobile phase was used as the diluent	Mobile phase was used as the diluent
Flow rate	1ml/min	1.0ml per min	1.0 ml per min	1.0 ml per min	1.0 ml per min
Column	symmetry ,C18 4.6x150mm ,5µm	Zodiacsil C18 4.6x150mm 5µm	Hypersil RPC8 4.5×150mm 5.0 µm	Zodiac sil RPC18 4.6×250mm 5µm	zodiac silRP C18 4.6×250mm 3.0µm
Detector wavelength	236nm	240 nm	240 nm	240 nm	240 nm
Column oven	Ambient	Ambient	Ambient	Ambient	Ambient
Injection volume	10µl	20µl	20µl	10µl	20µl
Running time	10min	8.0 min	10 min	5min	10min

Preparation of phosphate buffer [3]:
2.95 grams of KH2PO4and 5.45 grams of K2HPO4 was weighed and taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water and pH was adjusted to 3 with orthophosphoric acid. The resulting solution was sonicated and filtered.

Preparation of mobile phase:
Mix a mixture of above buffer 300 ml (30%) and 700 ml of methanol (HPLC grade-70%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

Preparation of the individual Imipenem standard preparation:
10 mg of Imipenem working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1.5 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of the individual Cilastatin standard preparation:
1 mg of Cilastatin working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 3 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of the Imipenem and Cilastatin standard and sample solution:

Sample solution preparation:
10 mg of Imipenem and 1 mg Cilastatin tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Standard solution preparation:
10 mg Imipenem and 1mg Cilastatin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Procedure:
10µL of the blank, standard and sample were injected into the chromatographic system and areas for the Imipenem and Cilastatin the peaks were used for calculating the % assay by using the formulae.
System suitability

Tailing factor for the peaks due to Imipenem and Cilastatin in standard solution should not be more than 1.5.

Theoretical plates for the Imipenem and Cilastatin peaks in standard solution should not be less than 2000.
Assay calculation

$$\boldsymbol{Assay}\ \% = \frac{\boldsymbol{samplearea}}{\boldsymbol{Standardarea}} \times \frac{\boldsymbol{dilutionsample}}{\boldsymbol{dilutionofstandard}} \times \frac{\boldsymbol{P}}{\mathbf{100}} \times \frac{\boldsymbol{Avg.wt}}{\boldsymbol{Lc}} \times \mathbf{100}$$

Where:
Avg.wt = average weight of tablets
P= Percentage purity of working standard
LC= Label Claim of Imipenem mg/ml.

ANALYTICAL METHOD VALIDATION [4-10]:
1. Specificity
The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

2. Linearity
Preparation of stock solution
10 mg of Imipenem and 1mg of Cilastatin working standard were accurately weighed and were transferred into a 10ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Preparation of Level – I (50ppm of Imipenem and 5 ppm of Cilastatin)
Preparation of Level – II (100ppm of Imipenem and 10ppm of Cilastatin)
Preparation of Level – III (150ppm of Imipenem and 15ppm of Cilastatin)

Preparation of Level – IV (200 ppm of Imipenem and 20ppm of Cilastatin)
Preparation of Level – V (250 ppm of Imipenem and 25ppm of Cilastatin)

Procedure
Each level was injected into the chromatographic system and peak area was measured. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and the correlation coefficient was calculated.
Correlation coefficient should be not less than 0.999.

3. Range
Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 50µg/ml-250µg/ml and 5µg/ml-25µg/ml of Imipenem and Cilastatin respectively.

4. Accuracy
Preparation of standard stock solution
10mg of Imipenem and 1mg of Cilastatin working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1 ml of the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of sample solutions
For preparation of 50% solution (with respect to target assay concentration)
5mg of Imipenem and 0.5 mg of Cilastatin working standard were accurately weighed and transferred into a 10 ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock Solution).Further pipette out 10 ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Procedure
The standard solutions of accuracy 50%, 100% and 150%were injected into chromatographic system. Calculate the amount found and amount added for Imipenem and Cilastatin and calculate the individual % recovery and mean % recovery values.

Acceptance criteria
The % recovery for each level should be between 98.0 to 102.0%

5. Precision
5.1 Repeatability
Preparation of stock solution
10 mg of Imipenem and 1 mg of Cilastatin working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted 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.

Acceptance criteria
The % RSD for the area of five standard injections results should not be more than 2.

5.2IntermediatePrecision/Ruggedness
To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on different days by using different make column of same dimensions.

Preparation of stock solution
10 mg of Imipenem and 1mg of Cilastatin working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted 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.

Acceptance criteria
The % RSD for the area of five sample injections results should not be more than 2%.

6. Limit of detection (LOD)
LOD's can be calculated based on the standard deviation of the response (SD)and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:
LOD=3.3 X σ/S
Where
σ - Standard deviation (SD)
S - Slope

7. Limit of quantification
LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.
Formula:

$$LOD=10 X \sigma/S$$

Where
σ - Standard deviation
S - Slope

8.Robustness
As part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method.

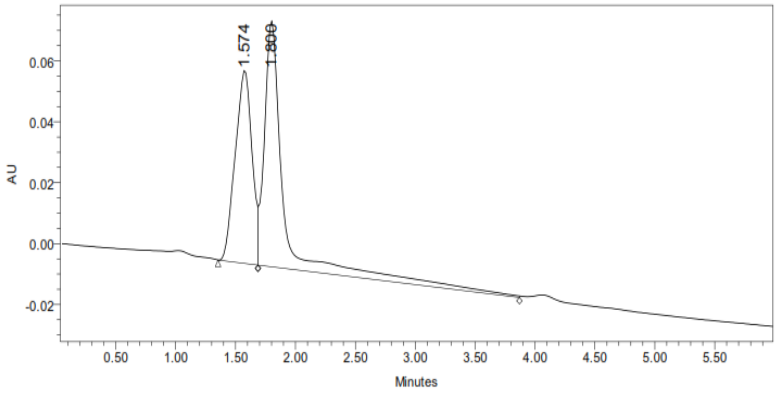
The flow rate was varied at 0.4ml/min to 0.6 ml/min. Standard solution 150 ppm of Imipenem and 15 ppm of Cilastatin was prepared and analysed using the varied flow rates along with method flow rate.

The organic composition in the mobile phase was varied from 65% to75 %standard solution 150 µg/ml of Imipenem and15 µg/ml of Cilastatin were prepared and analysed using the varied mobile phase composition along with the actual mobile phase composition in the method.

9. System suitability
10 mg of Imipenem and 1 mg of Cilastatin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of Imipenem and Cilastatin from the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

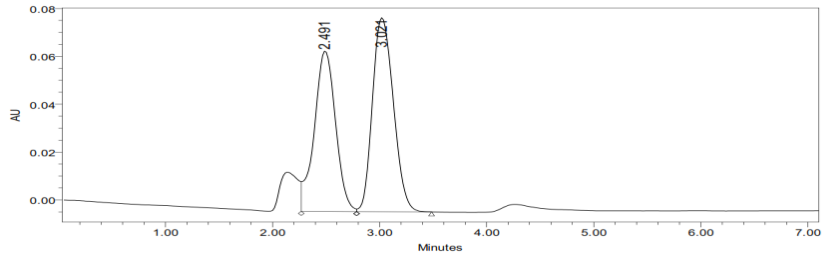
RESULTS AND DISCUSSIONS:

Chromatogram showing trial-1 injection



	RT	Area	Height	Injection	USP Plate Count	USP Resolution
1	1.574	613531	63337	1	469	
2	1.800	912828	80724	1	1040	0.9

Fig. Chromatogram showing trial-2 injection

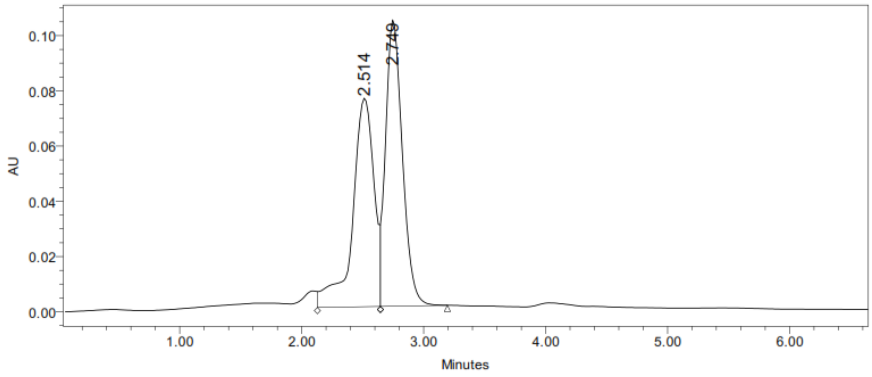


	RT	Area	Height	Injection	USP Plate Count	USP Resolution	USP Tailing
1	2.491	925902	67060	1	798		
2	3.021	1101490	81049	1	1120	1.5	1.18

Observation:

In this trial only Cilastatinpeak only eluted, still more trials were required for both peaks.

Chromatogram showing trial-3 injection

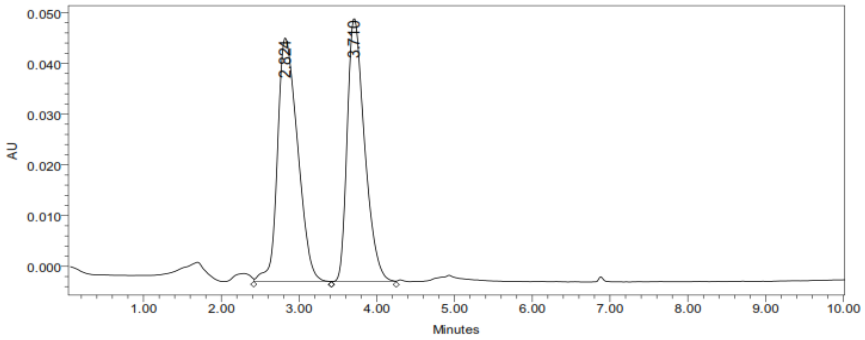


	RT	Area	Height	Injection	USP Plate Count	USP Resolution
1	2.514	923626	75553	1	1127	
2	2.749	992267	103822	1	1781	0.8

Observation:

In this trial both Imipenem and Cilastatin were eluted but there is no proper resolution. Still more trials were required for better resolution in peaks.

Chromatogram showing trial-4 injections

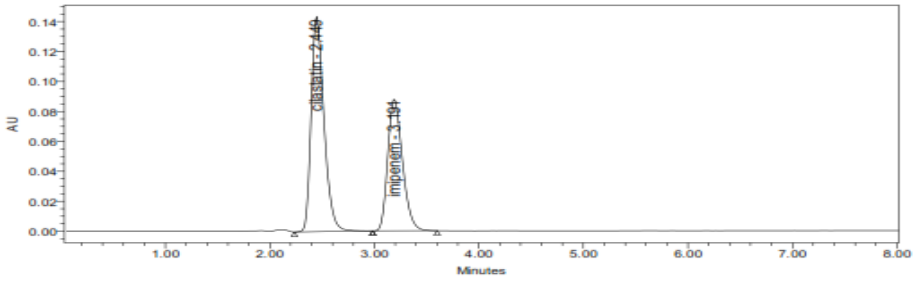


	RT	Area	Height	Injection	USP Plate Count	USP Resolution	USP Tailing
1	2.824	864805	48117	1	553		1.29
2	3.710	806629	51910	1	1290	2.0	1.43

Observation:

The separation was good; peak shape was good, still more trials were required to reduce the retention times of peaks.

Chromatogram showing trial-5 injection



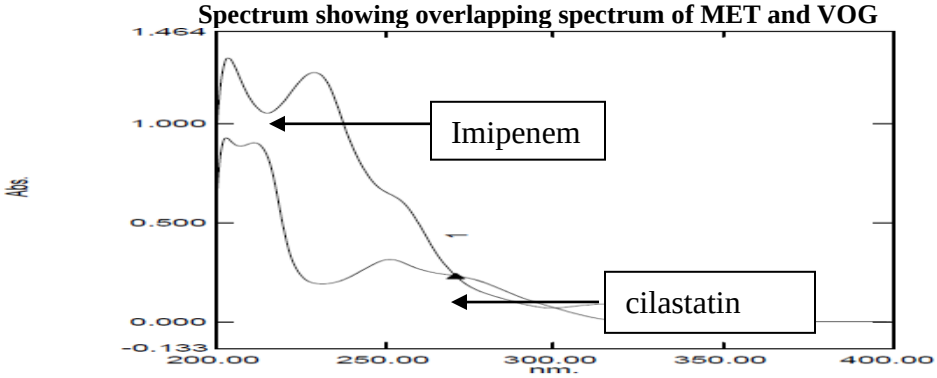
	Peak Name	RT	Area	Height	Injection	USP Plate Count	USP Resolution	USP Tailing	Symmetry Factor
1	cilastatin	2.449	1214356	143778	2	1939		1.32	1.32
2	imipenem	3.191	859991	88167	2	2439	3.0	1.26	1.26

Observation:

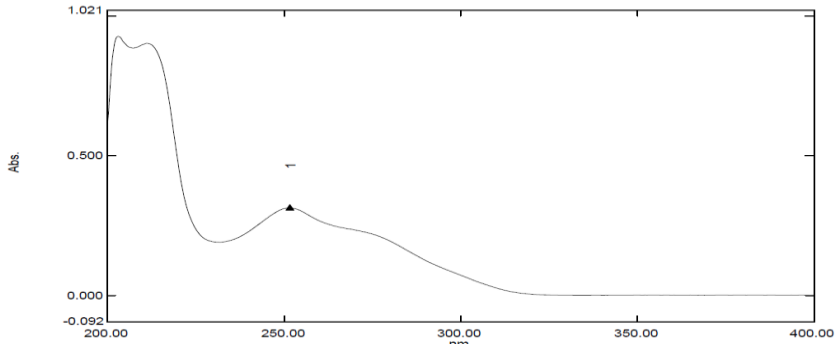
The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks,so it is taken as final method.

Method Development

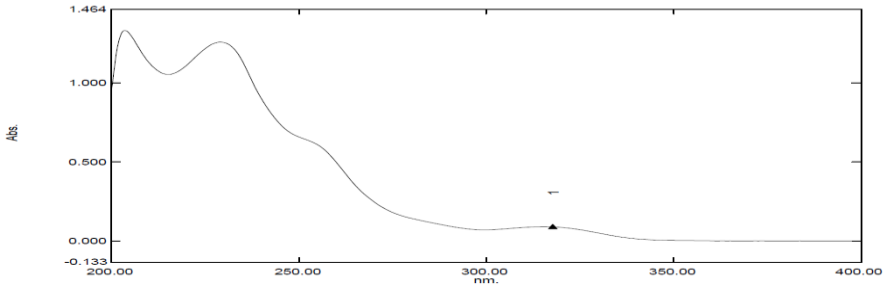
Spectrum showing overlapping spectrum of MET and VOG



Spectrum showing wavelength of cilastatin

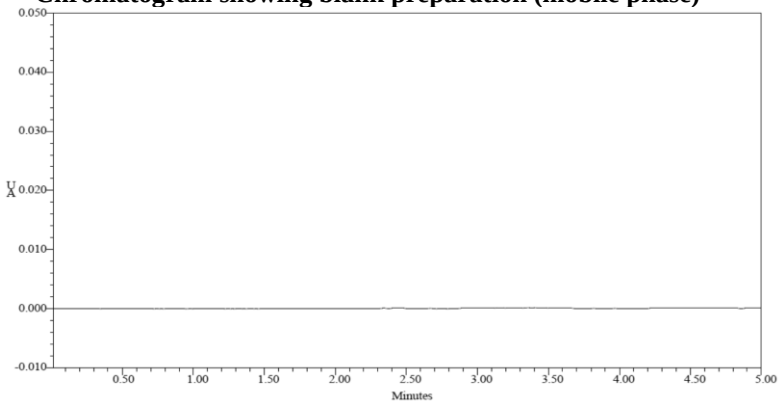


Spectrum showing wavelength of Imipenem



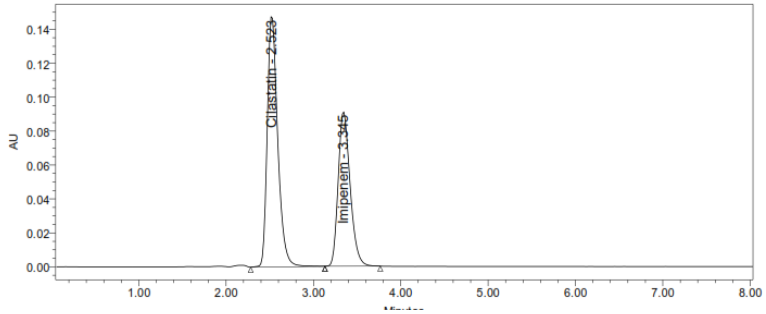
Optimized chromatographic conditions for simultaneous estimations of Imipenem and cilastatin by RP-HPLC method

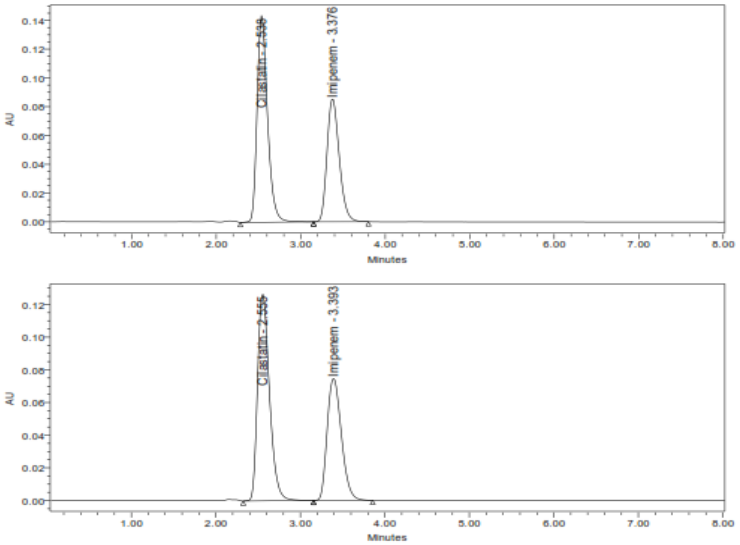
Chromatogram showing blank preparation (mobile phase)



Assay calculation for Imipenem and cilastatin

Chromatogram showing assay of sample injection-1,2,3





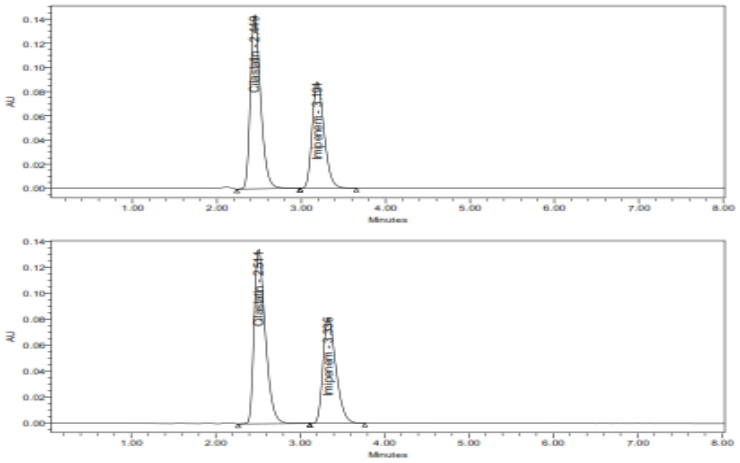
Peak Name: Cilastatin

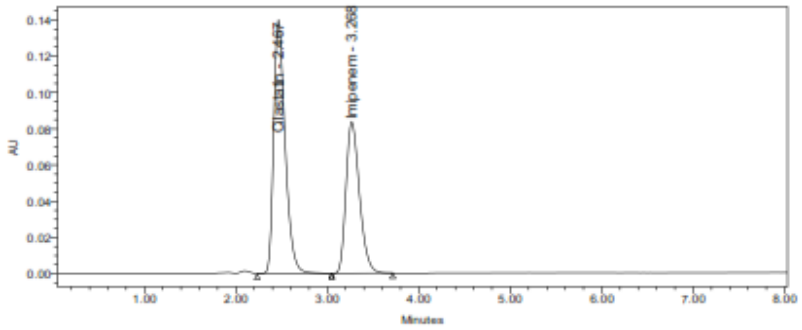
	Peak Name	RT	Area	Height	USP Plate Count	USP Tailing	Injection
1	Cilastatin	2.523	1225760	148888	2235.73	1.33	1
2	Cilastatin	2.538	1222608	143618	2104.27	1.30	2
3	Cilastatin	2.555	1234704	126626	1643.59	1.33	3
Mean			1227690.6				
Std. Dev.			6274.7				
% RSD			0.5				

Peak Name: Imipenem

	Peak Name	RT	Area	Height	USP Plate Count	USP Resolution	USP Tailing	Injection
1	Imipenem	3.345	866132	91030	2885.91	3.48	1.25	1
2	Imipenem	3.376	861910	85689	2602.43	3.37	1.23	2
3	Imipenem	3.393	871292	74645	1939.77	2.95	1.25	3
Mean			866444.7					
Std. Dev.			4698.7					
% RSD			0.5					

Chromatogram showingassay of standered injection -1,2,3.





Showing assay slandered results

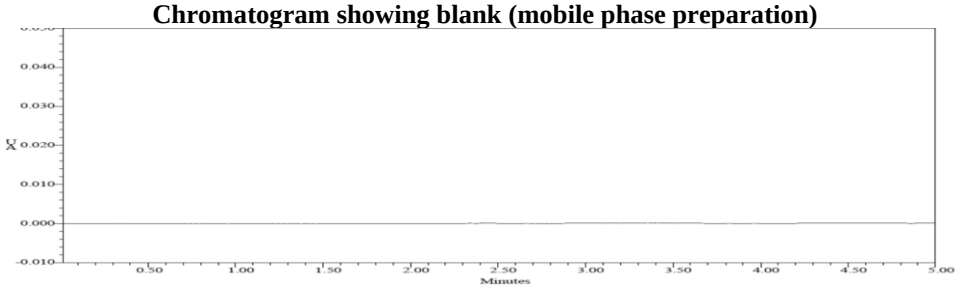
	Peak Name	RT	Area	Height	USP Plate Count	USP Resolution	USP Tailing
1	Cilastatin	2.449	1214356	143778	1938.96		1.32
2	Cilastatin	2.467	1217143	139906	1847.94		1.36
3	Cilastatin	2.511	1230473	134589	1741.58		1.43
4	Imipenem	3.191	861161	88187	2438.26	3.03	1.26
5	Imipenem	3.268	855169	83700	2358.16	3.13	1.30
6	Imipenem	3.336	868604	81477	2243.15	3.05	1.36

The retention time of Imipenem and cilastatin was found to be 3.345 mins and 2.523mins respectively. The system suitability parameters for Imipenem and cilastatin such as theoretical plates and tailing factor were found to be 2885, 1.25and 42235, 1.33. Resolution was 3.48. The % purity Imipenem and cilastatin in pharmaceutical dosage form was found to be 98.24 and 100.27% respectively.

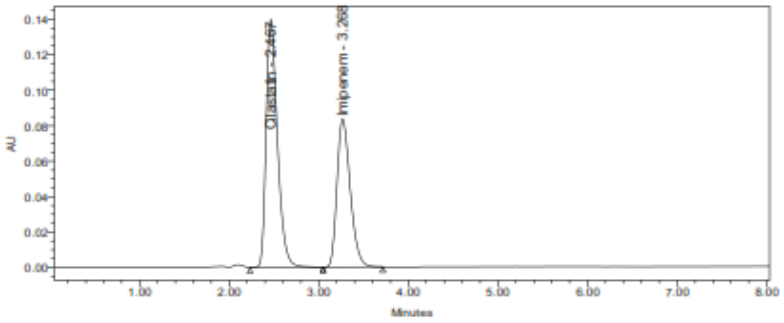
VALIDATION REPORT:

Specificity

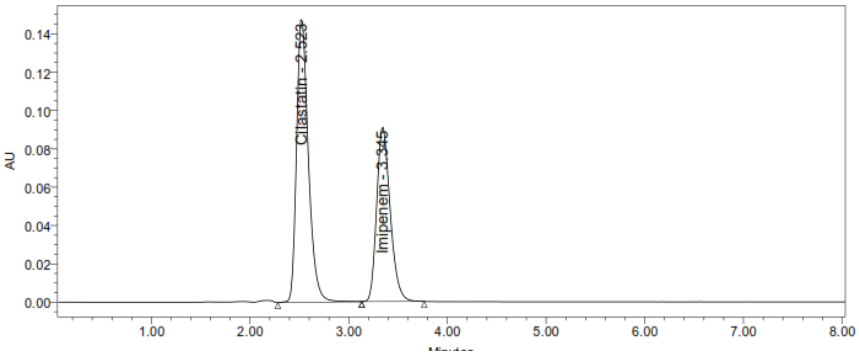
Chromatogram showing blank (mobile phase preparation)



Chromatogram showing standard injection



Chromatogram showing sample injection

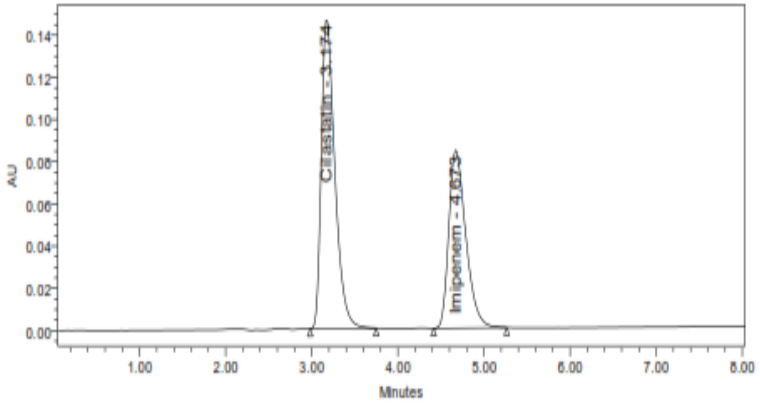


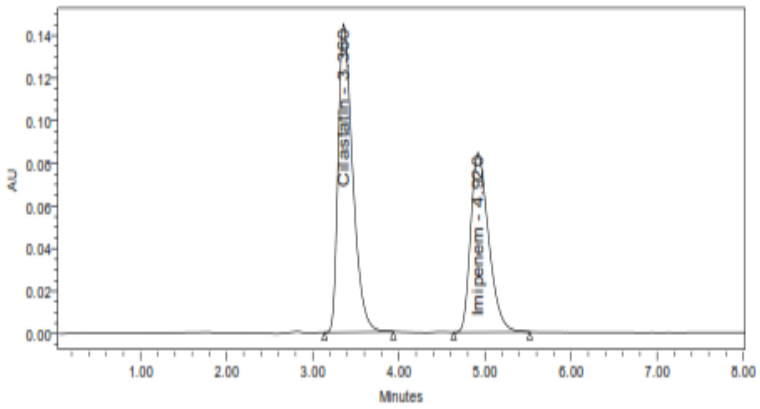
The specificity test was performed for Imipenem and cilastatin. It was found that there was no interference of impurities in retention time of analytical peak.

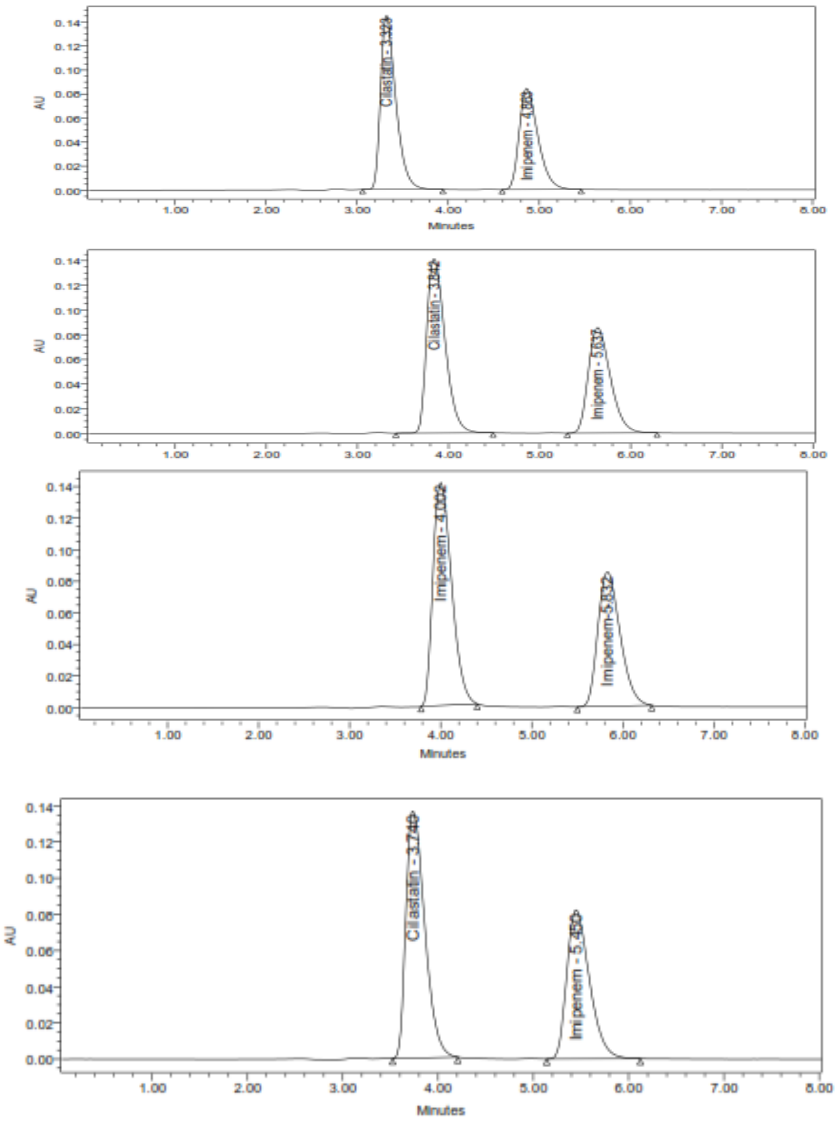
4.1.4.2. Linearity

The linearity study was performed for the concentration of 50 ppm to 250 ppm and 5ppm to 25 ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. The chromatograms are shown in below and results are tabulated in Table Calibration graph for imi and cilaare shown in

Chromatograms showing linearity level-1 to level 5 (50ppm-250 ppm of imiand 5ppm -50ppm of cila) injections.







Peak Name: Cilastatin

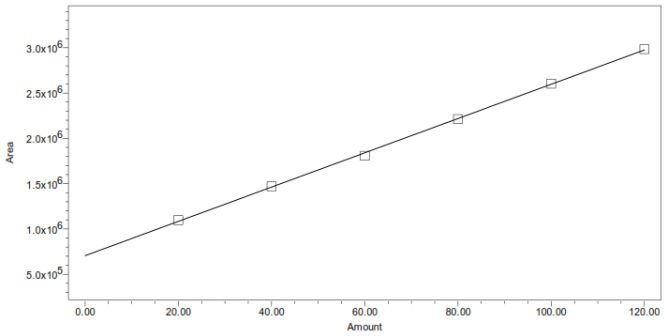
	Peak Name	RT	Area	Height	USP Plate Count	USP Tailing	Injection
1	Cilastatin	3.174	1592260	146364	2973.61	1.49	1
2	Cilastatin	3.174	2000516	147364	2973.61	1.49	2
3	Cilastatin	3.323	2420416	144812	2873.05	1.52	3
4	Cilastatin	3.360	2905813	145422	2912.90	1.52	4
5	Cilastatin	3.740	3270034	136518	2654.63	1.50	5
6	Cilastatin	3.842	3671138	141267	2898.11	1.43	6
Mean							
Std. Dev.							
% RSD							

Peak Name: Imipenem

	Peak Name	RT	Area	Height	USP Plate Count	USP Resolution	USP Tailing	Injection
1	Imipenem	4.002	1097254	141033	2852.74	4.51	1.37	5
2	Imipenem	4.673	1471214	84462	2672.17	4.42	1.38	1
3	Imipenem	4.863	1807502	84306	2557.54	4.39	1.40	3
4	Imipenem	4.920	2212400	84282	2627.38	4.24	1.38	2
5	Imipenem	5.450	2603215	82319	2444.22	4.36	1.38	6
6	Imipenem	5.637	2984663	85374	2506.06		1.32	4
Mean								
Std. Dev.								
% RSD								

Linearity Results for Imipenem:

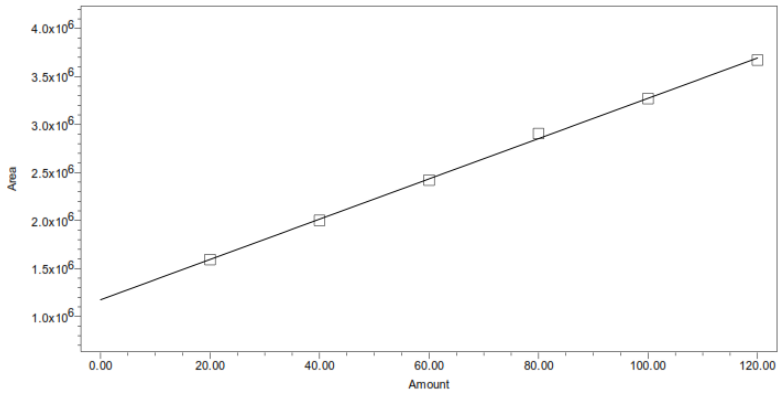
S.No	Linearity Level	Concentration	Area
1	I	50 ppm	471543
2	II	100 ppm	656277
3	III	150 ppm	794999
4	IV	200 ppm	946124
5	V	250 ppm	1002139
Correlation Coefficient			0.999



Imipenemr^2 = 0.999

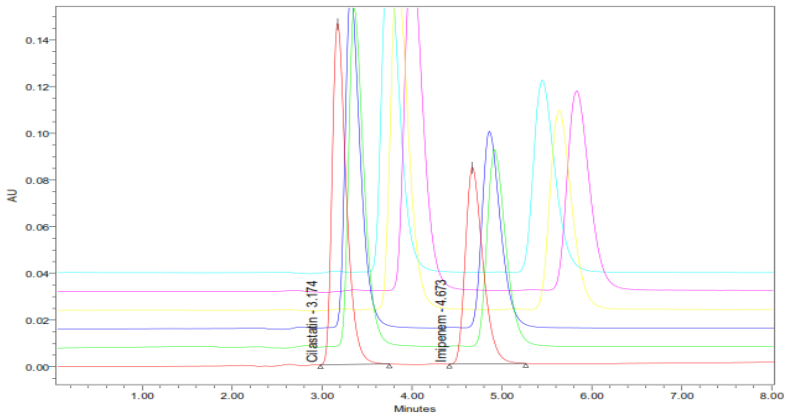
Linearity Results for CILASTATIN

Showing calibration graph for cilastatin



Cilastatin r^2 = 0.999

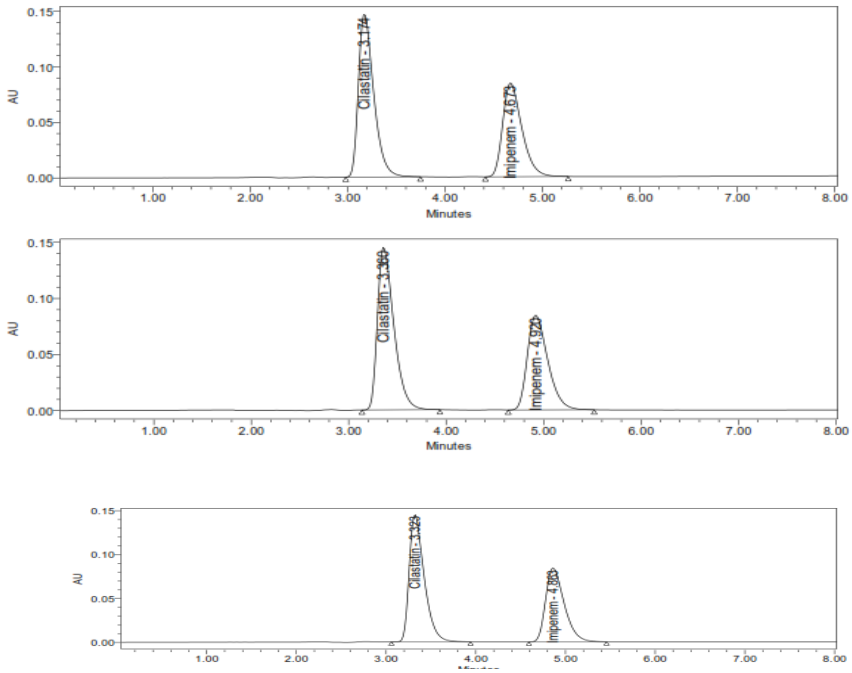
OVERLAY DIAGRAM



The linearity study was performed for concentration range of 50.µg-250µg and 5µg-50µg of Imipenem and cilastatin and the correlation coefficient was found to be 0.999 and 0.999.(NLT 0.999).

Accuracy

Chromatograms showingaccuracy-50% injection-1,2,3



Peak Name: Cilastatin

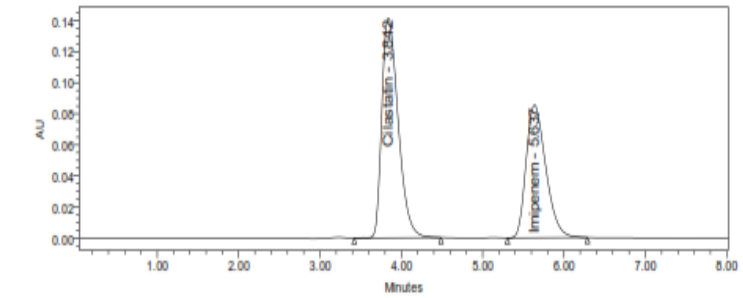
	Peak Name	RT	Area	Height	USP Plate Count	USP Tailing
1	Cilastatin	3.174	1592260	147364	2974	1.5
2	Cilastatin	3.323	1680416	144812	2873	1.5
3	Cilastatin	3.360	1695813	145422	2913	1.5
Mean			1656163			

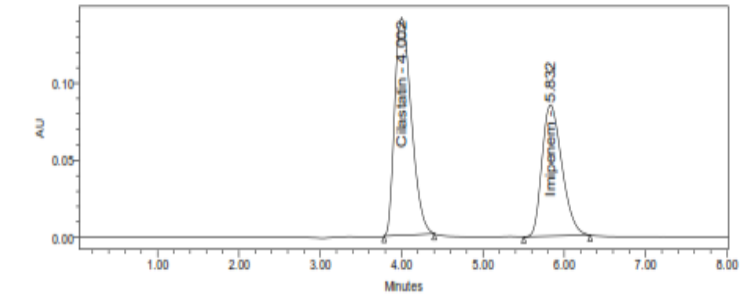
Peak Name: Imipenem

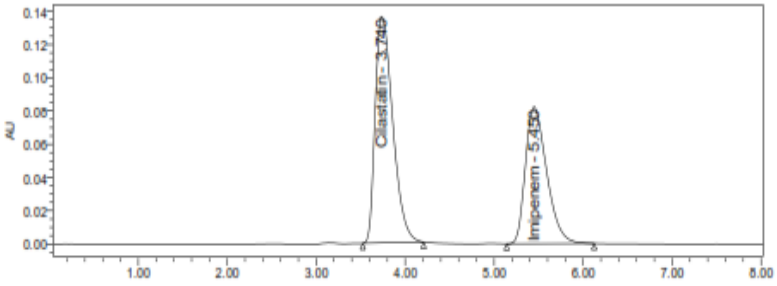
	Peak Name	RT	Area	Height	USP Plate Count	USP Resolution	USP Tailing
1	Imipenem	4.673	1151214	84462	2672	4.5	1.4
2	Imipenem	4.863	1207502	84306	2558	4.4	1.4
3	Imipenem	4.920	1222400	84282	2627	4.4	1.4
Mean			1193706				

Accuracy -100%

Chromatogram showing accuracy -100%injection-1,2,3.







Peak Name: Cilastatin

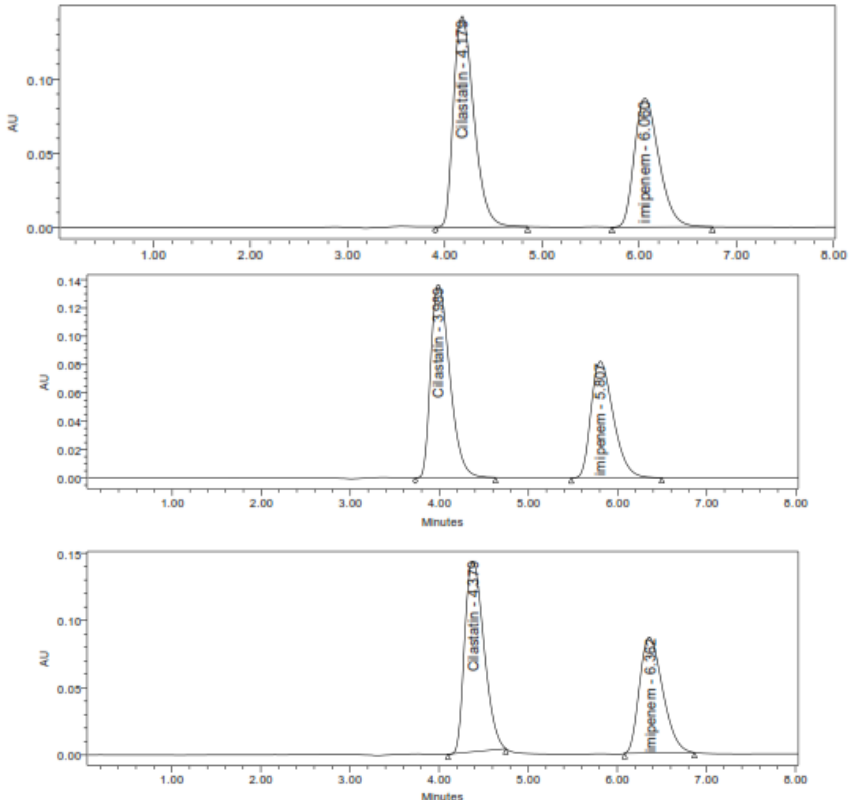
	Peak Name	RT	Area	Height	USP Plate Count	USP Tailing
1	Cilastatin	3.842	1870034	141033	2655	1.5
2	Cilastatin	4.002	1971138	133319	2898	1.4
3	Cilastatin	3.740	1920586	125374	2663	1.3
Mean						

Peak Name: Imipenem

	Peak Name	RT	Area	Height	USP Plate Count	USP Resolution	USP Tailing
1	Imipenem	5.637	1444622	141033	2853	4.2	1.4
2	Imipenem	5.832	1363215	82319	2444	4.4	1.4
3	Imipenem	5.450	1434663	85374	2506	4.6	1.3
Mean			1601711				

Accuracy 150%

Chromatogram showing accuracy -150 %injection-1,2,3.



Peak Name: Cilastatin

	Peak Name	RT	Area	Height	USP Plate Count	USP Tailing
1	Cilastatin	3.989	2029007	137187	2699	1.4
2	Cilastatin	4.379	2087327	142528	2699	1.3
3	Cilastatin	4.179	2058167	142528	2985	1.4
Mean						

Peak Name: imipenem

	Peak Name	RT	Area	Height	USP Plate Count	USP Resolution	USP Tailing
1	imipenem	5.807	1432827	82354	2471	4.3	1.4
2	imipenem	6.362	1527033	86883	2433	4.4	1.3
3	imipenem	6.060	1479930	85883	2733	4.4	1.3
Mean							

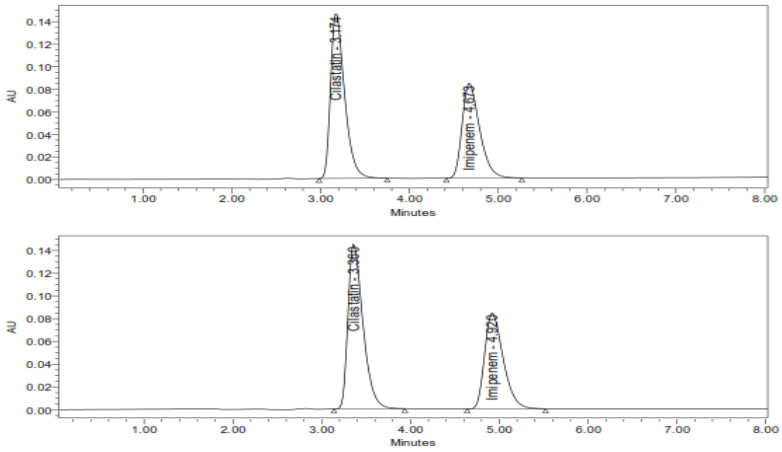
The accuracy study was performed for % recovery of Imipenem and cilastatin. The % recovery was found to be 99.18% and 99.91% respectively (NLT 98% and NMT 102%)

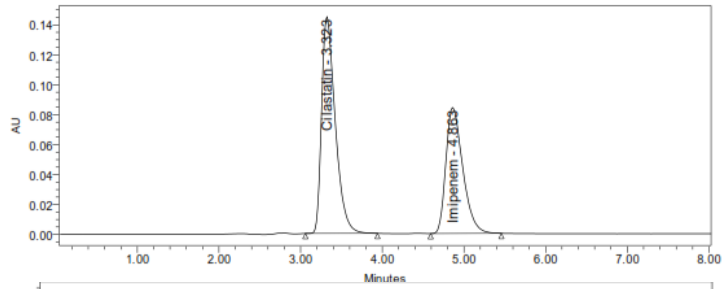
Precision

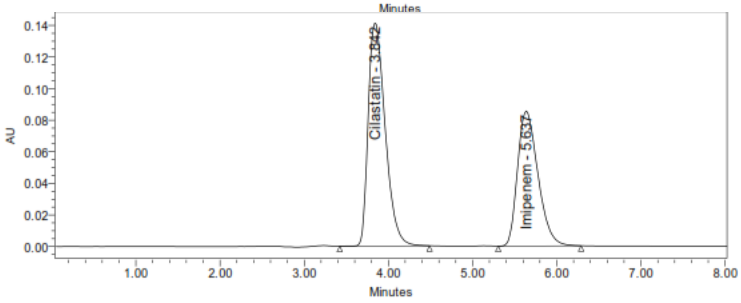
Repeatability

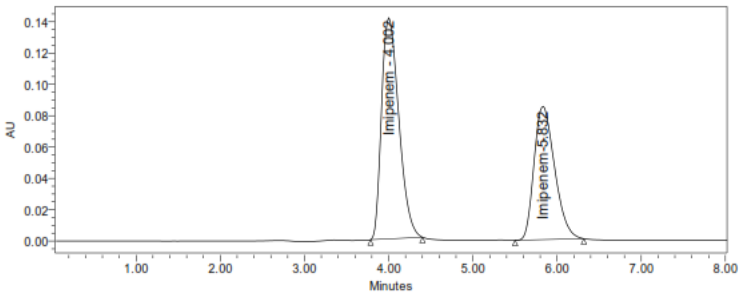
The precision study was performed for five injections of Imipenem and cilastatin. Each standard injection was injected into chromatographic system. The area of each Standard injection was used for calculation of % RSD.

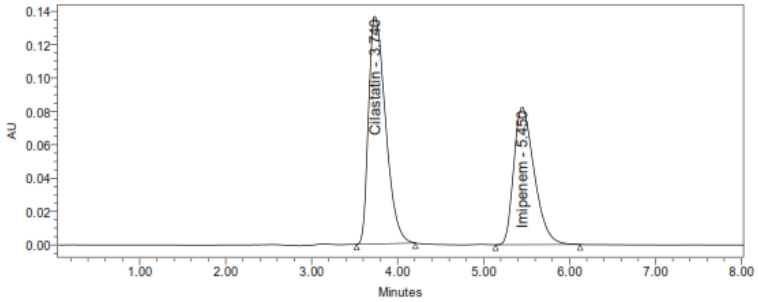
Chromatograms showing precisioninjections-1 to 5











Showing% RSD results for cilastatin and imipenem

Peak Name: Cilastatin

	Peak Name	RT	Area	Height	USP Plate Count	USP Tailing	Injection
1	Cilastatin	3.174	1592260	146364	2973.61	1.49	1
2	Cilastatin	3.174	1600516	147364	2973.61	1.49	2
3	Cilastatin	3.323	1620416	144812	2873.05	1.52	3
4	Cilastatin	3.360	1605813	145422	2912.90	1.52	4
5	Cilastatin	3.740	1670034	136518	2654.63	1.50	5
6	Cilastatin	3.842	1571138	141267	2898.11	1.43	6
Mean			1610030				
Std. Dev.			33600.6				
% RSD			2.0				

Peak Name: Imipenem

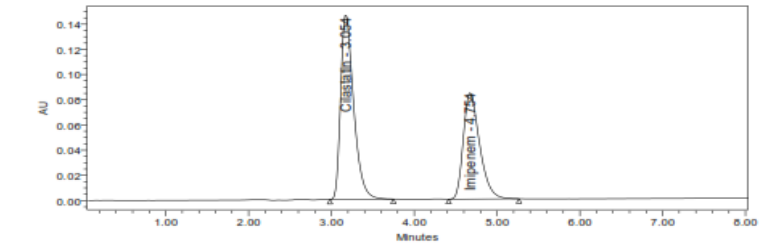
	Peak Name	RT	Area	Height	USP Plate Count	USP Resolution	USP Tailing	Injection
1	Imipenem	4.002	1197254	141033	2852.74	4.51	1.37	5
2	Imipenem	4.673	1171214	84462	2672.17	4.42	1.38	1
3	Imipenem	4.863	1207502	84306	2557.54	4.39	1.40	3
4	Imipenem	4.920	1212400	84282	2627.38	4.24	1.38	2
5	Imipenem	5.450	1203215	82319	2444.22	4.36	1.38	6
6	Imipenem	5.637	1184663	85374	2506.06		1.32	4
Mean			1196041.2					
Std. Dev.			15477.3					
% RSD			1.2					

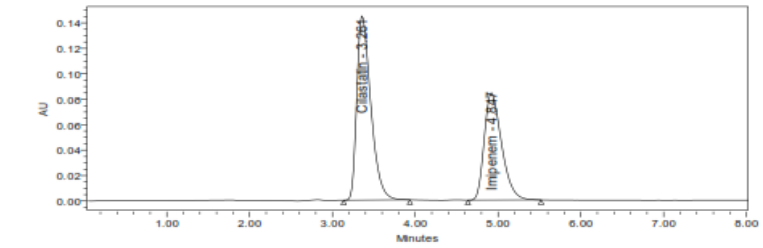
The Method precision study was performed for the %RSD of Imipenem and cilastatin was found to be 0.7 and 0.8(NMT 2).

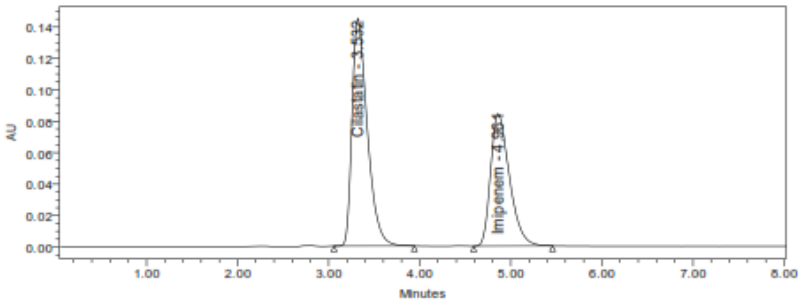
Intermediate precision/Ruggedness

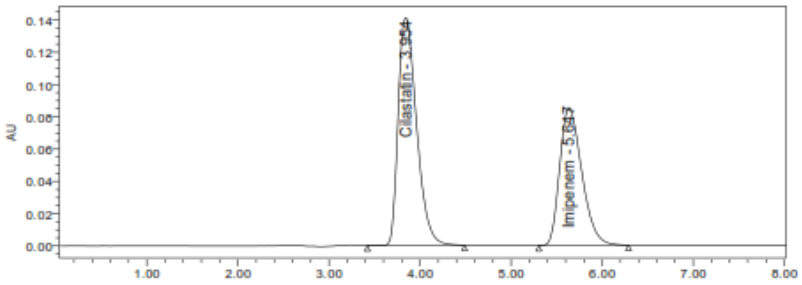
The intermediate precision study was performed for five injections of Imipenem and cilastatin. Each standard injection was injected into chromatographic system. The area of each standard injection was used for calculation of % RSD.

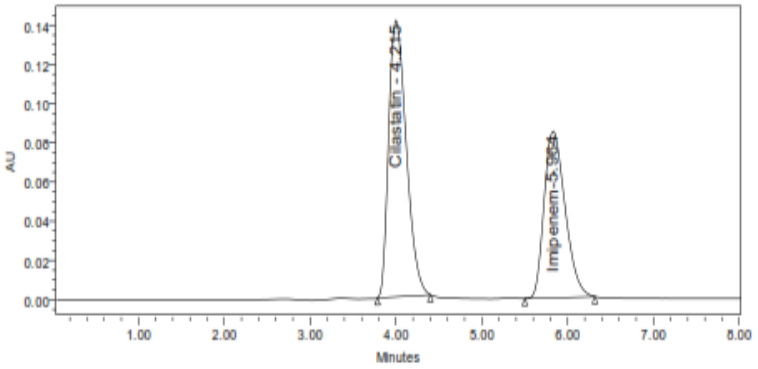
Chromatograms showing intermediate precision day1injections-1to 5

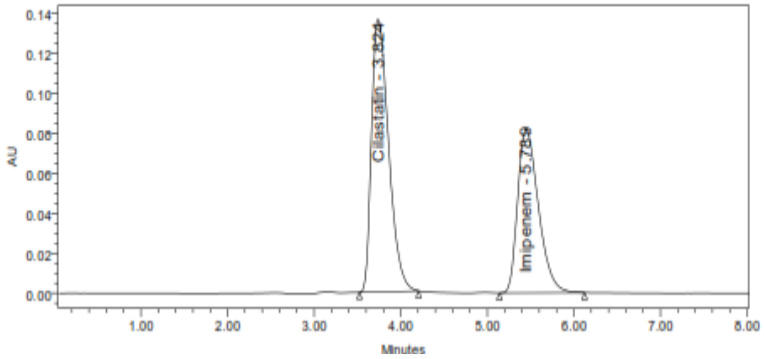












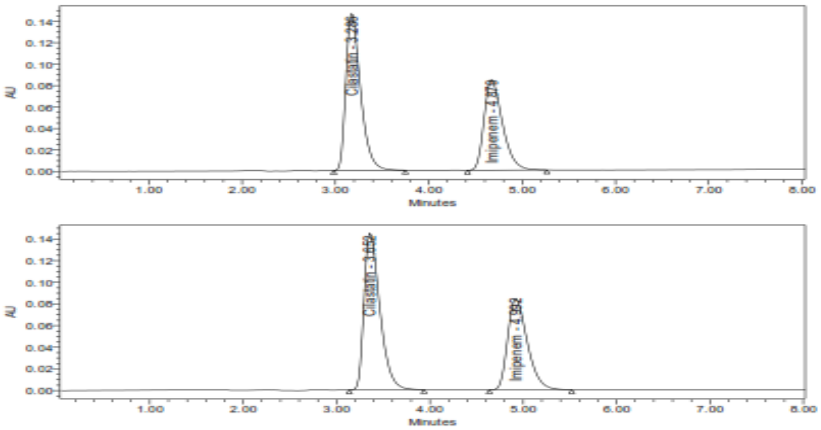
Peak Name: Cilastatin

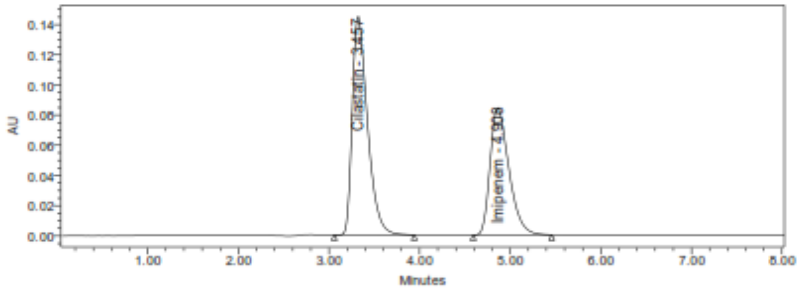
	Peak Name	RT	Area	Height	USP Plate Count	USP Tailing	Injection
1	Cilastatin	3.054	1747364	146364	2973.61	1.49	1
2	Cilastatin	3.261	1720516	147364	2973.61	1.49	2
3	Cilastatin	3.532	1690416	144812	2873.05	1.52	3
4	Cilastatin	3.954	1705813	145422	2912.90	1.52	4
5	Cilastatin	4.125	1710034	136518	2654.63	1.50	5
6	Cilastatin	3.824	1721138	141267	2898.11	1.43	6
Mean			1715880				
Std. Dev.			19101.4				
% RSD			1.1				

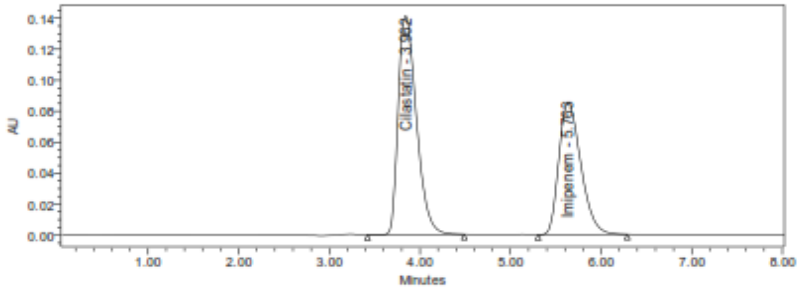
Peak Name: Imipenem

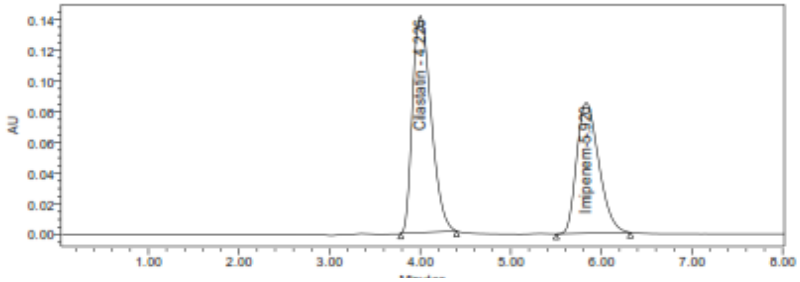
	Peak Name	RT	Area	Height	USP Plate Count	USP Resolution	USP Tailing	Injection
1	Imipenem	4.754	1637254	141033	2852.74	4.51	1.37	5
2	Imipenem	4.847	1621214	84462	2672.17	4.42	1.38	1
3	Imipenem	4.961	1657502	84306	2557.54	4.39	1.40	3
4	Imipenem	5.647	1602400	84282	2627.38	4.24	1.38	2
5	Imipenem	5.964	1643215	82319	2444.22	4.36	1.38	6
6	Imipenem	5.789	1634663	85374	2506.06		1.32	4
Mean			1632708					
Std. Dev.			18975.3					
% RSD			1.1					

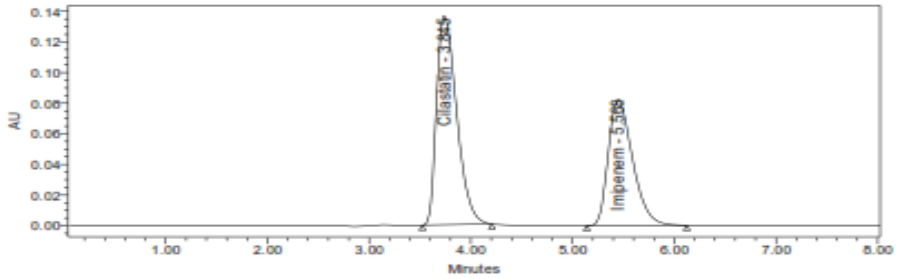
Day2




Cilastatin - 3.457
Imipenem - 4.908
AU
Minutes


Cilastatin - 3.982
Imipenem - 5.703
AU
Minutes


Cilastatin - 4.226
Imipenem-5.920
AU


Cilastatin - 3.845
Imipenem - 5.389
AU
Minutes

Peak Name: Cilastatin

	Peak Name	RT	Area	Height	USP Plate Count	USP Tailing	Injection
1	Cilastatin	3.286	4200051	146364	2973.61	1.49	1
2	Cilastatin	3.652	4120416	147364	2973.61	1.49	2
3	Cilastatin	3.457	4105613	144612	2673.05	1.52	3
4	Cilastatin	3.962	4170034	145422	2912.90	1.52	4
5	Cilastatin	4.226	4071136	136516	2654.63	1.50	5
6	Cilastatin	3.645	4110030	141267	2696.11	1.43	6
Mean			4129580				
Std. Dev.			47008.9				
% RSD			1.1				

Peak Name: Imipenem

	Peak Name	RT	Area	Height	USP Plate Count	USP Resolution	USP Tailing	Injection
1	Imipenem	4.879	2307254	141033	2852.74	4.51	1.37	5
2	Imipenem	4.992	2241214	64462	2672.17	4.42	1.36	1
3	Imipenem	4.908	2197502	64306	2557.54	4.39	1.40	3
4	Imipenem	5.763	2212400	64262	2627.36	4.24	1.36	2
5	Imipenem	5.920	2183215	62319	2444.22	4.36	1.36	6
6	Imipenem	5.568	2234663	65374	2506.06		1.32	4
Mean			2229375.2					
Std. Dev.			43972.8					
% RSD			1.9					

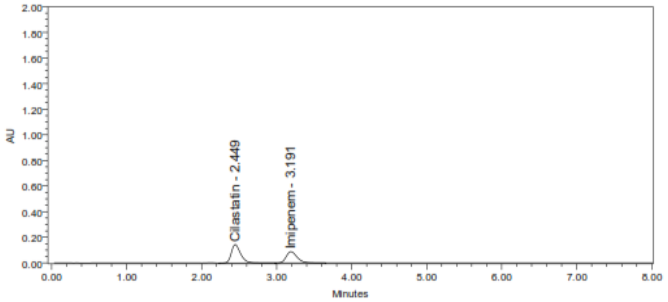
The intermediate precision was performed for %RSD of Imipenem and cilastatin was found to be 0.5 and 0.5 respectively (NMT 2).

Detection limit

LOD's can be calculated based on the standard deviation of the response (SD)and the slope of the calibration curve (S) at levels approximating the LOD according to the formula.The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:LOD $= 3.3 \text{ X } \frac{\sigma}{S}$

Where σ - Standard deviation (SD) S - Slope



Showing results for Limit of Detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Cilastatin	371827.90	563365963	2.17
Imipenem	5401.60	479884400	0.0372

The LOD was performed for Imipenem and cilastatin was found to be 2.17and 0.0372respectively.

Quantitation limit:

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:$LOQ = 10 \text{ X } \frac{\sigma}{S}$

Where σ - Standard deviation S - Slope

Showing results for Limit of Quantitation

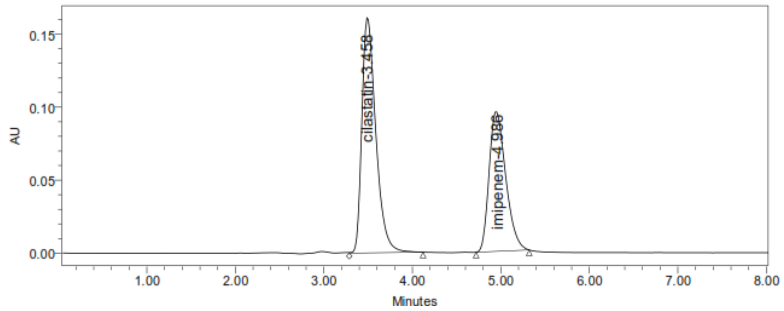
Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Cilastatin	371827.90	563365963	6.60
Imipenem	5401.60	479884400	0.112

The LOQ was performed forImipenem and cilastatin was found to be 6.60 and 0.112 respectively.

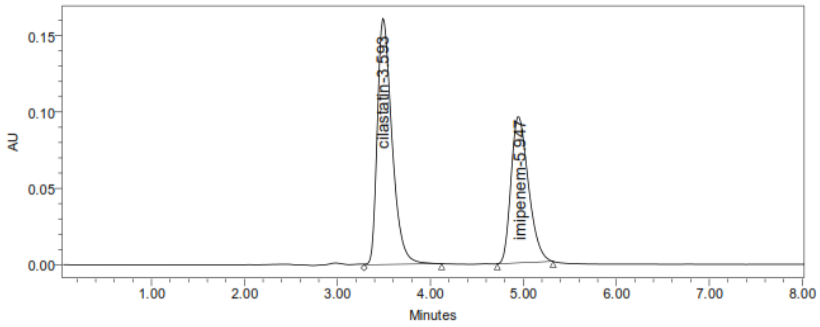
Robustness:

The robustness was performed for the flow rate variations from 0.4ml/min to 0.6ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Imipenem and cilastatin. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%.The chromatograms are shown in Fig. below and results are tabulated in Table. below.

Chromatogram showing more flow rate 0.8ml/min



Chromatogram showing less flow rate 1.2 ml/min



The results are summarized on evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±0.2ml/min. The method is robust only in less flow condition.

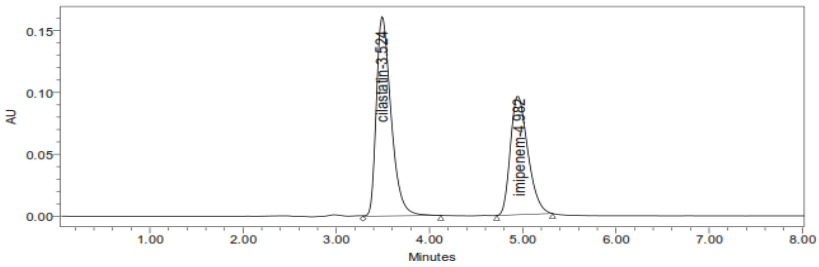
Showing system suitability results for cilastatin

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	2590	1.39
2	**1**	**2294**	**1.27**
3	1.2	2146	1.26

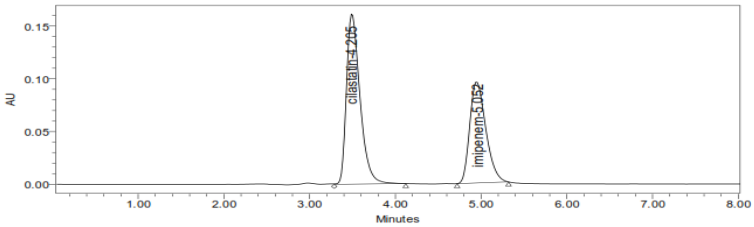
Showing system suitability results for Imipenem

S.No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	5435	1.04
2	**1**	**4891**	**1.03**
3	1.2	4781	1.04

Chromatogram showing more organic phase ratio



Chromatogram showing less organic phase ratio



On evaluation of the above results, it can be concluded that the variation in±5%. Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the mobile phase ±5%.

Showing system suitability results for cilastatin

S.No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	2347	1.44
2	***Actual**	**2294**	**1.27**
3	5 % more	2239	1.13

Showing system suitability results for Imipenem

S.No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	5437	0.99
2	***Actual**	**4891**	**1.03**
3	5 % more	4817	1.05

DISCUSSION:

A new method was recognized for simultaneous estimation of Imipenem and cilastatin by RP-HPLC method. The chromatographic circumstances were effectively developed for the separation of Imipenem and cilastatin by using SYMMETRYC18 column (4.6×150mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol : phosphate buffer($KH_2PO_4$and K_2HPO_4) pH 3 (pH was adjusted with orthophosphoric acid),detection wavelength was 258nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times was found to be 3.345 mins and 2.523 mins. The percentage purity of Imipenem and cilastatin was initiate to be 100.27% and 99.87% correspondingly. The system suitability parameters for Imipenem and cilastatin such as theoretical plates and tailing factor was found to be 2885, 1.25 and 2235 and 1.33, the resolution was found to be3.48. The analytical technique was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Imipenem and cilastatin found in concentration range of 50µg-250µg and 5µg-50µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery were found to be 99.56% and 99.48%, %RSD for repeatability was 1.2 and 2, % RSD for intermediate precision was 1.1 and 1.1 respectively. The precision study was precise, robust, and repeatable.LOD value was 0.03 and 2.17, and LOQ value was 0.11 and 6.6 respectively.

Hence the recommended RP-HPLC method can be used for repetitive analysis of Imipenem and cilastatinin API and Pharmaceutical dosage form.

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