Advances in Bioresearch
Adv. Biores., Vol 14 (6) November 2023: 173-184
©2023 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.14.6.173184

Advances in Bioresearch

ORIGINAL ARTICLE

Stability indicating HPLC method for the Simultaneous estimation of Imipenem, Relebactam and Cilastatin in Imipenem, Relebactam and Cilastatin powder for solution for infusion Dosage form

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ABSTRACT
The main aim and objective of the research work is to develop an effective, sensitive, economical and simple reverse phase HPLC method for the simultaneous estimation of Imipenem, Relebactam and Cilastatin in Imipenem, Relebactam and Cilastatin powder for solution for infusion dosage form. The separation was achieved by using column Phenomenex Luna InertClone ODS (250 x 4.6 mm, 3 µm) in mobile phase consisted of pH 6.4 phosphate buffer and acetonitrile in the ratio of 85:15 v/v. Elution mode was isocratic, flow rate was 0.8 mL/min, column oven temperature was maintained 35°C and sample cooler temperature was maintained 5°C the injection volume was 20 µL, and detection was performed at 210 nm using a photodiode array detector (PDA), Run time 40 minutes. The retention time of Imipenem, Relebactam and Cilastatin was noted to be 10.65 minutes 12.29 minutes and 26.67 minutes respectively. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible, and consistent.
Keywords: *Liquid Chromatography, Imipenem, Relebactam and Cilastatin, powder for solution for infusion, Simultaneous estimation, Forced degradation, Validation*

Received 24.05.2023 Revised 01.09.2023 Accepted 11.11.2023

How to cite this article:
P.Ramya S, P. Bharath, K. Madhavi, and D.Ramachandran. Stability indicating HPLC method for the simultaneous estimation of Imipenem, Relebactam and Cilastatin in Imipenem, Relebactam and Cilastatin powder for solution for infusion dosage form. Adv. Biores., Vol 12 (6) November 2023: 173-184.

INTRODUCTION

Imipenem:

The chemical name of Imipenem is (5R,6S)-3-[[2-(formimidoylamino)ethyl]thio]-6-[(R)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid. It corresponds to the molecular formula $C_{12}H_{17}N_3O_4S.H_2O$, its relative molecular mass is 317.37 and it has the structure shown in **Figure 1.** Imipenem monohydrate [1] is a white or almost white or pale-yellow powder, slightly hygroscopic substance. It is slightly soluble in water and methanol. The pKa values for Imipenem have been determined by aqueous acidic/basic Potentiometric titration at 25°C. The respective pKa1 and pKa2 are ~3.2 and ~9.9. The molecule has three chiral centres and is optically active. Imipenem has only one known crystal form.

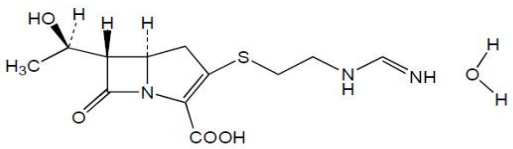

Fig. 1. Chemical structure of Imipenem monohydrate

Relebactam:

The chemical name of Relebactam hydrate is [(1R,2S,5R)-7-Oxo-2-(piperidin-1-ium-4-ylcarbamoyl)-1,6-diazabicyclo[3.2.1]octan-6-yl sulfate hydrate. It corresponds to the molecular formula $C_{12}H_{20}N_4O_6S.H2O$, its relative molecular mass is 366.4 and it has the structure shown in **Figure 2.** Relebactam [2] is a white to off-white hygroscopic crystalline powder. It is freely soluble in water, practically insoluble in isopropyl acetate, isopropyl alcohol, and acetonitrile and very slightly soluble in methanol. It has three stereogenic centers.

Fig. 2. Chemical structure of Relebactam monohydrate

Cilastatin:

The chemical name of Cilastatin is [Sodium (Z)-7-[[(R)-2-amino-2-carboxyethyl]thio]-2-[(S)-2,2-dimethylcyclopropanecarboxamido]-2-heptenoate. It corresponds to the molecular formula $C_{16}H_{25}N_2NaO_5S$, its relative molecular mass is 380.44 and it has the structure shown in **Figure 3.** Cilastatin [3] is a known active substance that has Ph. Eur. monograph available. Full information has been presented in the dossier concerning the manufacture of the active substance.

Cilastatin sodium is an off-white to white hygroscopic amorphous powder, which is very soluble in water and methanol. The molecule contains two chiral centers and is optically active. Cilastatin sodium is fully amorphous, and no crystal forms have been identified.

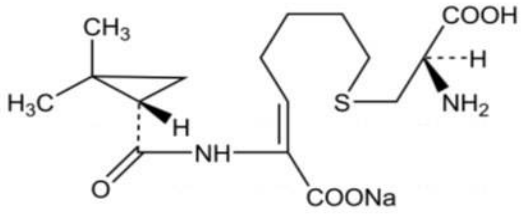

Fig. 3. Chemical structure of Cilastatin

The literature survey reveals that there are no HPLC methods were reported in major pharmacopoeias like USP, EP, JP and BP. Only few analytical methods were reported till date for the estimation of Imipenem and Cilastatin by using spectrophotometric [5-12] , RP-HPLC methods [13-20] and LC-MS methods [24-25].

Our aim to develop stability indicating HPLC method for the simultaneous estimation of Imipenem, Relebactam and Cilastatin in Imipenem, Relebactam and Cilastatin powder for solution for infusion dosage form. The present work describes a simple, stability indicating HPLC method for the simultaneous estimation of Imipenem, Relebactam and Cilastatin in Imipenem, Relebactam and Cilastatin powder for solution for infusion dosage form according to ICH guidelines [26-27].

MATERIAL AND METHODS

Chemicals and Reagents

Analytical-grade potassium dihydrogen orthophosphate, 1-hexane sulfonic acid sodium salt, orthophosphoric acid, Acetonitrile, Hydrochloric acid, Sodium hydroxide, Hydrogen peroxide and water, reagents and chemicals were procured from Merck Chemicals. Mumbai, India. Imipenem, Relebactam and Cilastatin standards were procured from Merck Chemicals. Mumbai, India.

Instruments and Equipment

Agilent HPLC model: 1200 infinity model with DAD, Bandelin ultrasonic bath, pH Meter (Thermo Orion Model) and Analytical Balance (Metller Toledo Model), Laboratory oven (Thermo Orion Model) were used in the present study.

Method of Analysis

Preparation of pH 6.4 phosphate buffer

Accurately weighed and transferred 2.7215 gm of potassium dihydrogen ortho phosphate and 2.1054 gm of 1-hexane sulfonic acid sodium salt was dissolved in 1000 mL of milli Q water and adjusted the pH to

6.4 with diluted sodium hydroxide solution. Filtered the solution with 0.45 µm membrane filter and sonicate to degas.

Preparation of mobile phase

Prepared a mixture of 850 mL of pH 6.4 phosphate buffer and 150 mL of acetonitrile in the ratio of 85:15 (%volume/volume). Filtered the solution with 0.45 µm membrane filter and sonicate to degas.

Preparation of diluent

Prepared a mixture of 500 mL of pH 6.4 phosphate buffer and 500 mL of acetonitrile in the ratio of 50:50 (%volume/volume). Filtered the solution with 0.45 µm membrane filter and sonicate to degas.

Preparation of standard

Weighed accurately and transferred 20.16 mg of Imipenem working standard, 10.38 mg of Relebactam working standard and 20.21 mg of Cilastatin working standard into a 100 mL volumetric flask. Added 50 mL of diluent sonicated to dissolve and diluted to volume with the same.

Preparation of sample solution

Reconstituted 2 vials (sample) with 20 mL of diluent and transferred the entire contents to 200 mL volumetric flask with suitable hypodermic needle and syringe. Rinsed the each vial with 20 mL diluent for 2 times and transfer the entire contents to same 200 mL volumetric flask with suitable hypodermic needle and syringe. Diluted to volume with diluent and mixed well. Transferred 2.0 mL of above sample solution in to 50 mL volumetric flask. Diluted to volume with diluent and mixed well.

Preparation of placebo solution

Reconstituted 2 vials (placebo) with 20 mL of diluent and transferred the entire contents to 200 mL volumetric flask with suitable hypodermic needle and syringe. Rinsed the each vial with 20 mL diluent for 2 times and transfer the entire contents to same 200 mL volumetric flask with suitable hypodermic needle and syringe. Diluted to volume with diluent and mixed well. Transferred 2.0 mL of above sample solution in to 50 mL volumetric flask. Diluted to volume with diluent and mixed well.

Method expansion and optimization of chromatographic conditions

The maximum UV absorbance (max) of the Imipenem, Relebactam and Cilastatin medicinal materials was observed at 210 nm, in accordance with UV-spectroscopic examination. Dissimilar mobile phases and Dissimilar column stationary phases were used to create a desirable peak shape in order to produce a suitable and reliable HPLC approach for the strength of Imipenem, Relebactam and Cilastatin in Imipenem, Relebactam and Cilastatin dose form.

Optimized chromatographic conditions

The separation was achieved by using column Phenomenex Luna InertClone ODS (250 x 4.6 mm, 3 µm) in mobile phase consisted of pH 6.4 phosphate buffer and acetonitrile in the ratio of 85:15 v/v. Elution mode was isocratic, flow rate was 0.8 mL/min, column oven temperature was maintained 35°C and sample cooler temperature was maintained 5°C the injection volume was 20 µL, and detection was performed at 210 nm using a photodiode array detector (PDA), Run time 40 minutes.

RESULTS

System suitability

As per the proposed method chromatographic conditions were set and mobile phase allowed equilibrating with stationary phase. Five replicate injections of standard solution were injected and the chromatograms were recorded for the drugs.

Table 1. System suitability results

Name of the Component	Retention Time	Theoretical plates	Tailing factor
Imipenem	10.72	5341	1.1
Relebactam	12.36	20545	1.0
Cilastatin	26.70	21685	1.2

Specificity

A study to establish the interference of blank and placebo were conducted. Diluent and placebo were introduced keen on the chromatograph in the individual above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of blank solution Figure 4. showed no peak at the retention time of Imipenem, Relebactam and Cilastatin peaks. This indicates that the diluent solution used in sample preparation does not interfere with the inference of Imipenem, Relebactam and Cilastatin in Imipenem, Relebactam and Cilastatin powder for solution for infusion dosage form. Similarly chromatogram of the placebo solution Figure 5. showed no peaks at the retention time of Imipenem,

Relebactam and Cilastatin peaks. This indicates that the placebo used in sample preparation does not interfere with the inference of Imipenem, Relebactam and Cilastatin in Imipenem, Relebactam and Cilastatin powder for solution for infusion dosage form. Similarly chromatogram of the standard and sample solution Figure 6 and 7.

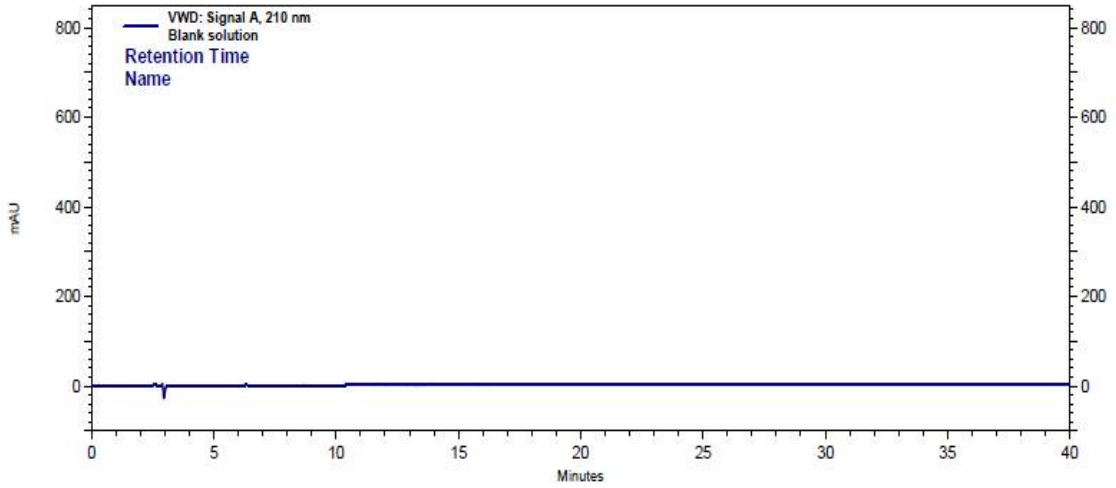

Fig 4. Typical chromatogram of blank

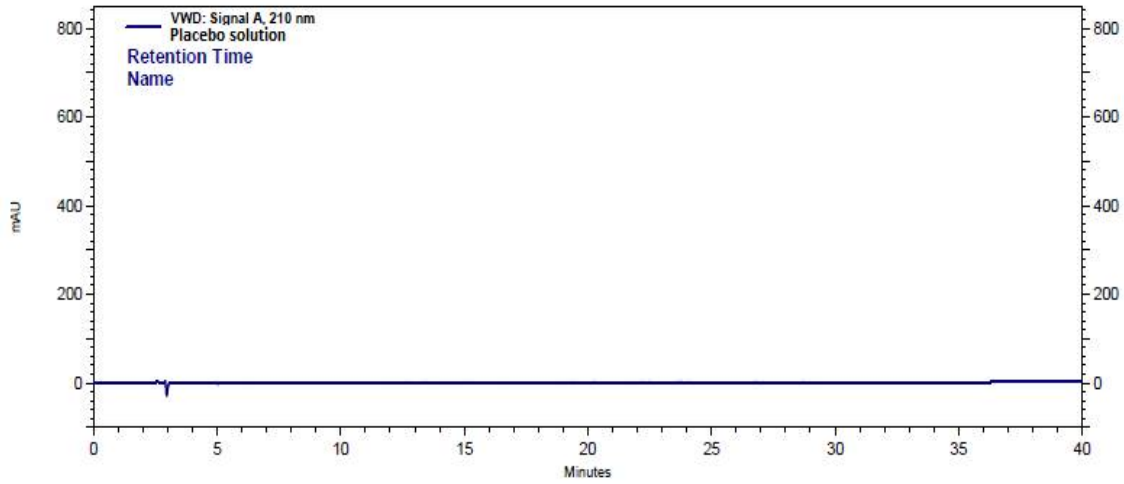

Fig. 5. Typical chromatogram of placebo

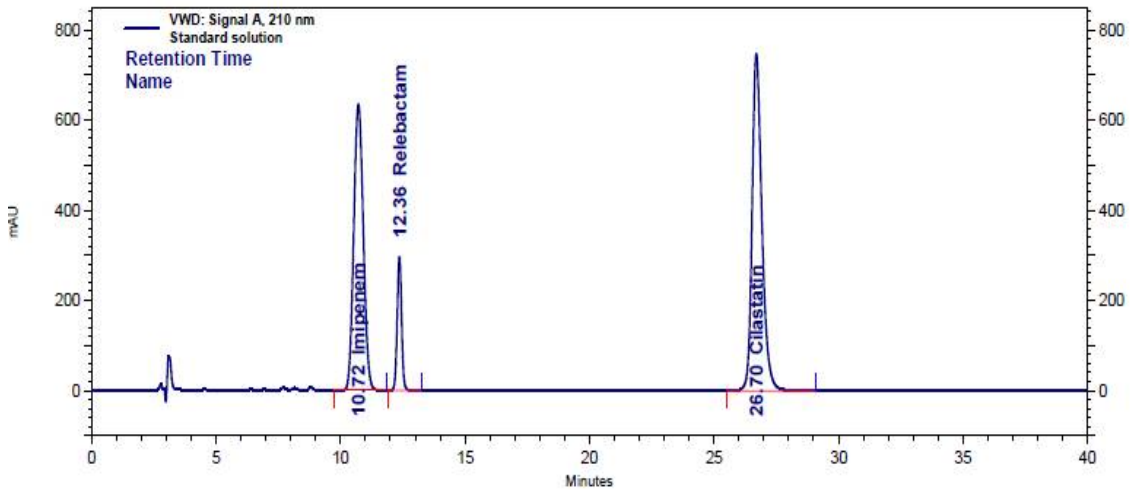

Fig. 6. Typical chromatogram of standard

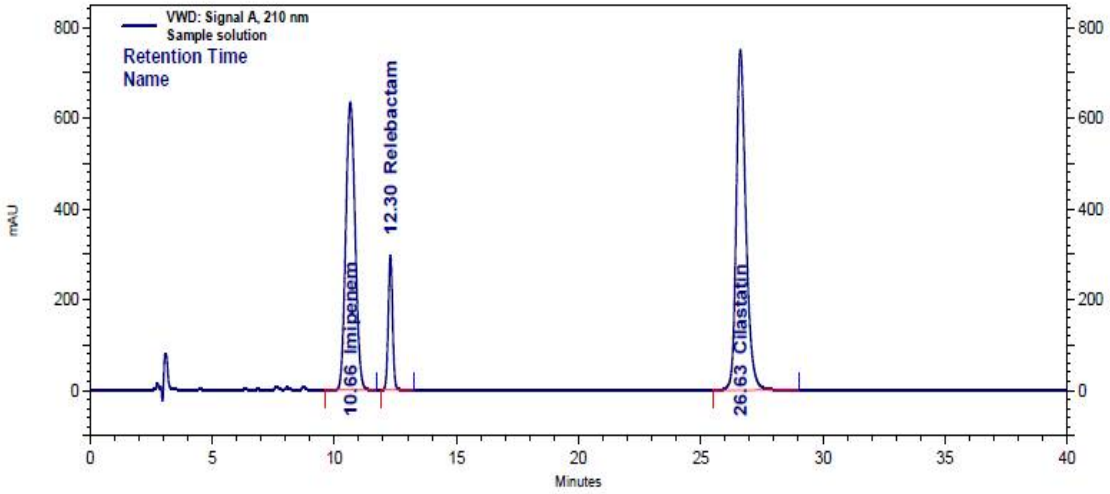

Fig. 7. Typical chromatogram of sample

Table 2. Specificity results

S.No	Name of the Component	Retention Time (min)	Blank	Placebo
1	Blank	ND	NA	NA
2	Placebo solution	ND	NA	NA
3	Standard solution			
	Imipenem	10.72	No	No
	Relebactam	12.36	No	No
	Cilastatin	26.70	No	No
4	Sample solution			
	Imipenem	10.66	No	No
	Relebactam	12.30	No	No
	Cilastatin	26.63	No	No

Force Degradation studies

A study was carry out to demonstrate the successful parting of degradants/impurities as of Enzalutamide. Separate portions of sample and placebo solutions were exposed to the following stress conditions to induce degradation. Stressed and unstressed samples were injected into the HPLC system with a PDA detector. The degradation study results were presented in Table 3. No significant degradation was observed in the Imipenem and Cilastatin. Significant degradation was observed in Relebactam in the acid, alkali and minor degradation was observed oxidation stress conditions. Hence it can be concluded that Relebactam is responsive to acid, alkali and oxidation.

Table 3. Forced Degradation Results

Stress condition	Degradation condition	%Assay Imipenem	%Assay Relebactam	%Assay Cilastatin	% Degradation
As such	Control sample	100.2	100.1	99.1	NA
Acid	0.5 N HCl/60°C/12 hrs	99.5	92.7	100.0	8.7
Alkali	0.5 N NaOH/60°C/12 hrs	99.7	93.8	100.3	6.4
Oxidative	3.0% H_2O_2/BT/ 12 hrs	99.1	95.4	99.8	4.7
Photolytic	1.2 million Lux hours or 200 watt hours/m^2 for 7 days	99.5	99.9	100.2	1.6
Humidity	90% RH Exposed for 2 days	100.0	100.1	99.5	1.2
Thermal	105°C/2 days	100.1	99.8	99.6	1.5

System precision

The standard solution was prepared as per the method, injected into on the HPLC system six times, and calculated the % RSD for the area responses. The statistics were revealed in Table 4.

Table 4. System precision results

S.No.	Imipenem	Relebactam	Cilastatin
Injection-1	3933481	5412895	6702739
Injection-2	3844561	5380214	6602516
Injection-3	3945128	5396184	6514855
Injection-4	3795068	5348129	6458692
Injection-5	3848265	5341765	6514862
Injection-6	3897851	5365478	6699753
Mean	3877392	5374111	6582236
%RSD	1.50	0.51	1.57

The relative standard deviation of six replicates standard solution consequences were establish to be within the specification limit i.e.1.50%, 0.51% and 1.57%.

Method precision

The method precision of the test technique was estimated by doing an assay for six samples of Imipenem, Relebactam and Cilastatin powder for solution for infusion (500 mg + 250 mg + 500 mg) as per the test technique. The content in mg and % label claim for Imipenem, Relebactam and Cilastatin for each of the test preparation was calculated. The middling content of the six arrangements and % RSD for the six observations were determined. The statistics were revealed in Table 5. Overall and individual % of Assay are complying as per test technique specification. The relative standard deviation of six assay preparations is 0.53%, 0.56% and 0.44%.

Table 5. Method precision results

S.No	No. of Preparations	% Assay Imipenem	% Assay Relebactam	% Assay Cilastatin
1	Preparation 1	100.2	100.1	99.1
2	Preparation 2	99.3	100.3	100.2
3	Preparation 3	100.1	99.1	99.7
4	Preparation 4	99.8	100.5	99.9
5	Preparation 5	100.8	99.3	100.1
6	Preparation 6	100.5	100.0	100.3
Average		100.1	99.9	99.9
SD		0.5269	0.5601	0.4401
%RSD		0.53	0.56	0.44

Linearity

Standard stock solution was diluted with diluent to get the final concentration of standard Imipenem, Relebactam and Cilastatin in the range of 50.4-302.4 µg/mL, 25.95-155.70 µg/mL and 50.525-303.15 µg/mL. Standard solutions of different concentration were injected separately and the chromatograms were recorded. Peak areas were recorded for each injected concentration of drugs and the calibration curves, concentration vs. peak area were constructed for the drugs. Linearity performance parameters are shown in results. The statistical data's for Imipenem, Relebactam and Cilastatin are shown in results. The statistics were revealed in Table 6, Table 7 and Table 8.

Table 6. Linearity data of Imipenem

S.No	Linearity Level	Concentration (ppm)	Area response
1	Linearity at 25%	50.4	951363
2	Linearity at 50%	100.8	1922726
3	Linearity at 75%	151.2	2814088
4	Linearity at 100%	201.6	3845457
5	Linearity at 120%	252.0	4806814
6	Linearity at 150%	302.4	5778177
Correlation coefficient (r^2)			0.9998
Intercept			-28666.1333
Slope			19171.0334
% Y-intercept			-0.74

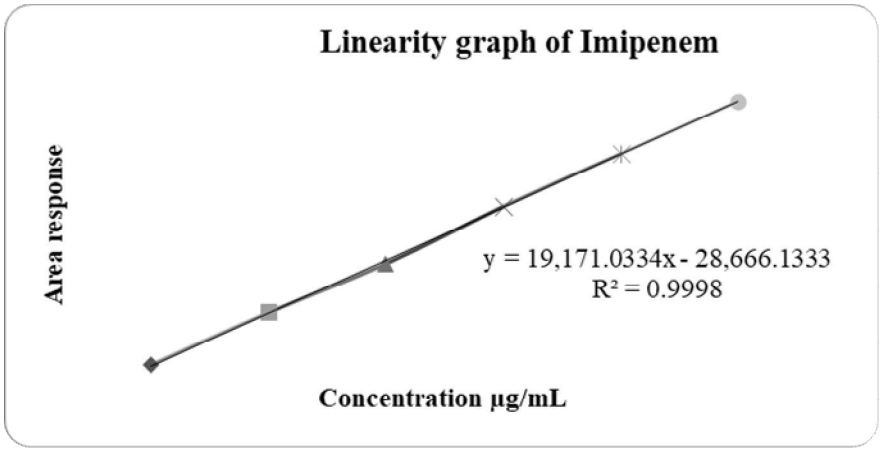

Fig. 8. Linearity graph of Imipenem

Table 7. Linearity data of Relebactam

S.No	Linearity Level	Concentration (ppm)	Area response
1	Linearity at 25%	25.95	1291195
2	Linearity at 50%	51.90	2582387
3	Linearity at 75%	77.85	4023576
4	Linearity at 100%	103.80	5364258
5	Linearity at 120%	129.75	6705951
6	Linearity at 150%	155.70	7997139
Correlation coefficient (r^2)			0.9998
Intercept			-63358.4
Slope			52013.3157
% Y-intercept			-1.2

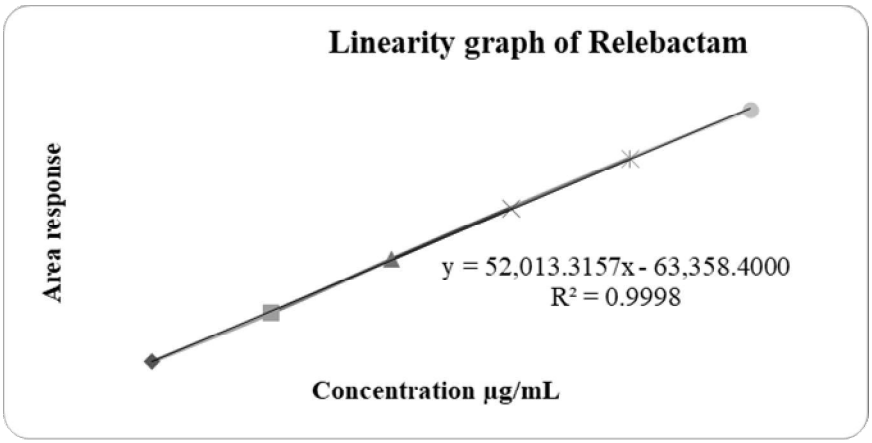

Fig. 9. Linearity curve of Relebactam

Table 8. Linearity data of Cilastatin

S.No	Linearity Level	Concentration (ppm)	Area response
1	Linearity at 25%	50.525	1639741
2	Linearity at 50%	101.050	3259975
3	Linearity at 75%	151.575	4893254
4	Linearity at 100%	202.100	6557782
5	Linearity at 120%	252.625	8197654
6	Linearity at 150%	303.150	9772244
Correlation coefficient (r^2)			0.9999
Intercept			6100.3333
Slope			32312.1962
% Y-intercept			0.1

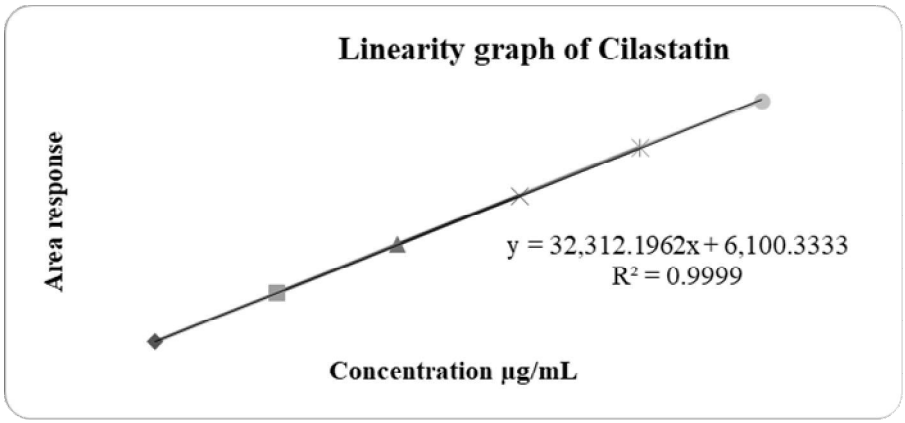

Fig. 10. Linearity curve of Cilastatin

Accuracy

A series of solutions were prepared by spiking the placebo and API in the range of about 50% to 150% of test concentration in triplicate and injected into HPLC system and analyzed as per the test method. Calculated individual % recovery and mean % recovery at each level the results are tabulated in Table 9 Table 10 and Table 11.

Table 9 Table 10 and Table 11 illustrates that the accuracy at 50% level, 100% level and 150% level for Imipenem, Relebactam and Cilastatin is meeting the acceptance criteria. From the above results, it is concluded that method is accurate.

Table 9. Accuracy results for Imipenem

S. No.	% Spike level	Amount added (µg)	Amount recovered (µg)	% Recovery	% Mean recovery
1	50	100.43	100.14	99.71	99.5
2		100.95	100.15	99.21	
3		100.41	100.11	99.7	
4	100	200.22	200.09	99.94	100
5		200.96	200.89	99.97	
6		200.16	200.26	100.05	
7	150	300.28	300.05	99.92	99.9
8		300.57	300.11	99.85	
9		300.45	300.19	99.91	

Table 10. Accuracy results for Relebactam

S. No.	% Spike level	Amount added (µg)	Amount recovered (µg)	% Recovery	% Mean recovery
1	50	50.42	50.51	100.18	100.3
2		50.37	50.58	100.42	
3		50.14	50.35	100.42	
4	100	100.28	100.31	100.03	100
5		100.41	100.45	100.04	
6		100.22	100.29	100.07	
7	150	150.82	150.44	99.75	99.7
8		150.77	150.33	99.71	
9		150.61	150.07	99.64	

Table 11. Accuracy data of Cilastatin

S. No.	% Spike level	Amount added (µg)	Amount recovered (µg)	% Recovery	% Mean recovery
1	50	100.09	100.23	100.14	100.3
2		100.21	100.35	100.14	
3		100.15	100.66	100.51	
4	100	200.47	200.51	100.02	100.1
5		200.32	200.49	100.08	
6		200.44	200.65	100.10	
7	150	300.29	300.35	100.02	100.0
8		300.34	300.41	100.02	
9		300.22	300.36	100.05	

Solution stability of analytical solutions

Standard and sample solutions were kept for 24 hrs at bench top (room temperature) and at refrigerator 2-8°C. The solution stability of standard and sample solutions was determined by comparison of old prepared standard solutions with freshly prepared standard solutions. The observations are tabulated below Table 12 to Table 15.

Table 12. Solution stability of standard at Bench top at RT

Condition	Name of the Component	Similarity factor		
		Initial	12 hrs	24 hrs
Room temperature	Imipenem	1.01	1.01	1.01
	Relebactam	1.01	1.01	1.00
	Cilastatin	1.01	1.02	1.01

Table 13. Solution stability of standard at Refrigerator

Condition	Name of the Component	Similarity factor		
		Initial	12 hrs	24 hrs
Refrigerator	Imipenem	1.01	1.01	1.01
	Relebactam	1.00	1.00	1.00
	Cilastatin	1.01	1.01	1.02

Table 14. Solution stability of sample at Bench top

Condition	Name of the Component	%Assay Initial	%Assay 12 hrs	%Assay difference	%Assay 24 hrs	%Assay difference
Room temperature	Imipenem	100.2	100.6	0.4	100.9	0.7
	Relebactam	100.1	100.5	0.4	100.9	0.8
	Cilastatin	99.1	99.6	0.5	100.1	1.0

Table 15. Solution stability of sample at refrigerator

Condition	Name of the Component	%Assay Initial	%Assay 12 hrs	%Assay difference	%Assay 24 hrs	%Assay difference
Refrigerator	Imipenem	100.2	100.3	0.1	100.3	0.1
	Relebactam	100.1	100.3	0.2	100.4	0.3
	Cilastatin	99.1	99.3	0.2	99.5	0.4

Table 12 to Table 15 illustrates that the solution stability of standard and sample solutions at different time intervals studied, from the above results, it is concluded that standard, and sample solutions are stable up to 24 hours in both the conditions (bench top and refrigerator).

Filter validation

Performed the filter validation for sample solution, one portion of the solution was centrifuged and the other portion of the solution was filtered through 0.45 µm PVDF and 0.45 µm Nylon filters. The observations are tabulated below Table 16.

Table 16. Results for Filter validation

Component Name	Filter Type	Area Response	Difference
Imipenem	Centrifuged sample	3875654	NA
	0.45 µm PVDF Filtered Sample	3861753	0.4
	0.45 µm Nylon Filtered Sample	3865941	0.3
Relebactam	Centrifuged sample	5375148	NA
	0.45 µm PVDF Filtered Sample	5341674	0.6
	0.45 µm Nylon Filtered Sample	5337460	0.7
Cilastatin	Centrifuged sample	6585846	NA
	0.45 µm PVDF Filtered Sample	6498321	1.3
	0.45 µm Nylon Filtered Sample	6448874	2.1

Table 16 illustrates that the filter validation study for sample solution with different filters (0.45 µm PVDF filters and 0.45 µm Nylon filters) compared with unfiltered sample solution (centrifuged). Based on the above results the filtered samples solutions are compatible for both 0.45 µm PVDF & 0.45 µm Nylon filters.

DISCUSSION

A simple, economic, accurate and precise HPLC method was successfully developed. In this method it was carried out by using Phenomenex Luna InertClone ODS (250 x 4.6 mm, 3 µm) and the mobile phase consisted of pH 6.4 phosphate buffer and acetonitrile in the ratio of 85:15 v/v. The flow rate is 0.8 mL/min. The column temperature was maintained at 35°C and sample temperature was maintained at 5°C, injection volume 20 µL and wavelength fixed at 210 nm, run time 40 minutes. The results obtained were accurate and reproducible. The method developed was statistically validated in terms of selectivity, accuracy, linearity, precision, stability of solution and filter study. For selectivity, the chromatograms

were recorded for standard and sample solutions of Imipenem, Relebactam and Cilastatin. Selectivity studies reveal that the peaks are well separated from each other. Therefore the method is selective for the simultaneous estimation of Imipenem, Relebactam and Cilastatin in Imipenem, Relebactam and Cilastatin powder for solution for infusion dosage form. There is no interference of diluent and placebo at Imipenem, Relebactam and Cilastatin peaks. The elution order and the retention times of standard preparations and sample preparations are comparable. Degradation study results were shown no significant degradation was observed in the Imipenem and Cilastatin. Significant degradation was observed in Relebactam in the acid, alkali and minor degradation was observed oxidation stress conditions. Hence it can be concluded that Relebactam is responsive to acid, alkali and oxidation. The results proved that the developed method has good selectivity and specificity. For system precision studies six replicate injections were performed. %RSD was determined from the peak areas of Imipenem, Relebactam and Cilastatin. The acceptance limit should be no more than 2.0%, and the results were found to be within the acceptance limits. For method precision studies six sample preparations were performed. The % assay for Imipenem, Relebactam and Cilastatin for each of the test preparation was calculated. The average % assay of the six preparations and % RSD for the six observations were determined. The acceptance limit should be not more than 2.0%, and the results were found to be within the acceptance limits. The linearity results for Imipenem, Relebactam and Cilastatin in the specified concentration range 50.4-302.4 µg/mL, 25.95-155.70 µg/mL and 50.525-303.15 µg/mL are found satisfactory, with a correlation coefficient greater than 0.99. Calibration curve was plotted and correlation co-efficient for Imipenem, Relebactam and Cilastatin found to be 0.9998, 0.9998 and 0.9999 respectively. The accuracy studies were shown as % recovery for Imipenem, Relebactam and Cilastatin at 50%-150% level. The limit of % recovered shown is in the range of 50% and 150% and the results obtained were found to be within the limits. Hence the method was found to be accurate. Solution stability parameter was established, standard and sample solutions are stable upto 24 hrs at bench top and at refrigerator.
Filter validation parameter was established and the filtered sample solutions are compatible for both 0.45 µm PVDF & 0.45 µm Nylon filters.

CONCLUSION

The developed method was validated for various parameters as per ICH guidelines like accuracy, precision, linearity, specificity, system suitability, solution stability and robustness. The results obtained were within the acceptance criteria. So, it can be concluded that the developed method is simple, precise, cost-effective, eco-friendly, and safe and can be successfully employed for the routine analysis of Imipenem, Relebactam and Cilastatin in Imipenem, Relebactam and Cilastatin powder for solution for infusion dosage form.

ACKNOWLEDGMENT

The authors are grateful to Department of Chemistry, University College of Sciences, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur. Andhra Pradesh, India, for providing the facilities to carry this research work.

FUNDING ACKNOWLEDGMENT

No funding was received.

CONFLICT OF INTERESTS

The authors claim that there is no conflict of interest.

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