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A New Simple Analytical Development and Validation of Imipenem and Cilastatin by Simultaneous Estimation of Pharmaceutical Dosage Form by RP-HPLC

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Abstract
A simple, rapid, precise, accurate and sensitive reverse phase liquid chromatographic method has been developed for the determination of imipenem and cilastatin by simultaneous estimation of pharmaceutical dosage form. The chromatographic method was standardized using X-Terra C18, 4.6×250mm 5.0µm i.d. column with UV detection at 238nm and mobile phase with the composition of phosphate buffer Ph 3.0: Acetonitrile with (50:50) ratio at a flow rate of 1.0 ml/min. The proposed method was successfully applied to the determination of imipenem and cilastatin by simultaneous estimation of pharmaceutical dosage form. The method was linear and accurate in the range of 50ppm-250ppm of Imipenem and Cilastatin. The recovery was in the range of 98% to 102% and limit of detection of Imipenem and Cilastatin was found to be 2.17µg/ml and 0.037µg/ml and quantification was found to be 6.60 µg/ml and 0.112µg/ml. Different analytical performance parameters such as precision, accuracy, limit of detection, limit of quantification and robustness were determined according to International Conference on Harmonization (ICH) guidelines.

Keywords
RP-HPLC, Imipenem and Cilastatin, Method development and validation, ICH Guidelines.

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INTRODUCTION:
Imipenem acts as an antimicrobial through the inhibition of cell wall synthesis of various gram-positive and gram-negative bacteria. This inhibition of cell wall synthesis in gram-negative bacteria is attained by binding to pencillin binding proteins (PBPs). Cilastatin is a specific and reversible renal dehydropeptidase-I inhibitor. Since the antibiotic, imipenem, is hydrolysed by dehydropeptidase-I, which resides in the brush border of the renal tubule, cilastatin is administered with imipenem to block the metabolism and thus the inactivation of imipenem so that antibacterial levels of imipenem can be attained in the urine. The drug also prevents the metabolism of leukotriene D4 to leukotriene E4 through the inhibition of leukotriene D4 dipeptidase. Ibrutinib

was estimated individually by few methods like simple HPLC1, UPLC2, LC-MS3 method validation of Imipenem and cilastatin. The objective of the work is to develop RP-HPLC method for the determination of imipenem and cilastatin by simultaneous estimation of pharmaceutical dosage form with simple , rapid, accurate and economical methods and validated for system suitability, linearity, accuracy, precision, robustness and stability of sample solution as per ICH guidelines.[1].It is an intra venous route administered, The objective of the work is to develop RP-HPLC method for estimation of Imipenem and cilastatin in tablet dosage form with simple , rapid, accurate and economical methods and validated for system suitability, linearity, accuracy, precision, robustness and stability of sample solution as per ICH guidelines.

The IUPAC Name of Imepenam is (5R,6S)-3-({2-[(E)-(amino methylidene) amino] ethyl} sulfanyl)-6-[(1R)-1-hydroxyethyl]-7-oxo-1-azabicyclo [3.2.0] hept-2-ene-2-carboxylic acid.[4]

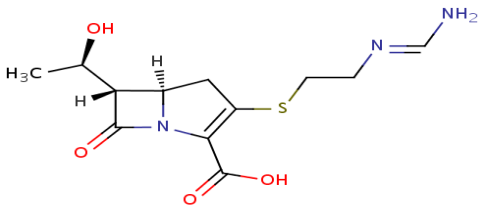


Fig 1: Chemical Structure of Imepenam

The IUPAC Name of cilastatin is (2Z)-7-{[(2R)-2-amino-2-carboxyethyl] sulfanyl}-2-{[(1S)-2,2-dimethylcyclopropyl] formamido} hept-2-enoic acid

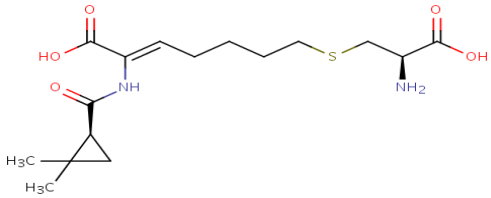


Fig 2: Chemical Structure of cilastatin

MATERIALS AND METHODS

HPLC Instrumentation & Conditions: The HPLC system employed was HPLC with Empower2 Software with Isocratic with UV-Visible Detector.

Standard and sample preparation for UV-spectrophotometer analysis: 10 mg of Imipenem and cilastatin standard was transferred into 10 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.1 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase. The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent. (After optimization of all conditions) for UV analysis. it scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Imipenem and cilastatin, so that the same wave number can be utilized in HPLC UV detector for estimating the Imipenem and cilastatin. While scanning the Imipenem and cilastatin solution we observed the maxima at 238nm. The UV spectrum has been recorded on ELICO SL-159 make UV – Vis spectrophotometer model UV-2450[5,6]. The scanned UV spectrum is attached in the following page.

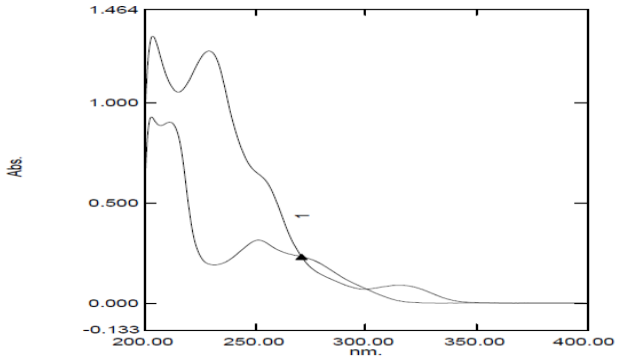


Fig 3: Overlapping spectrum of IMIPENEM and CILASTATIN UV spectrum

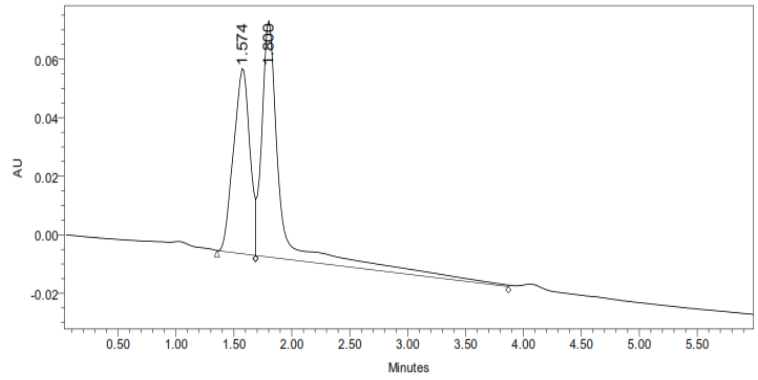
Optimized Chromatographic Conditions:
Column: X-Terra C18, 250x 4.6 mm 5.0 µm
Mobile Phase: Acn**:** phosphate buffer pH 3.0in the ratio 50:50(v/v)
Flow Rate: 1.0ml/minute
Wavelength: 238nm
Injection volume: 10µl
Run time: 10.0 mins.
Column temperature: 25°c
Sampler cooler: Ambient
MOBILE PHASE PREPARATION
Buffer Preparation:
2.95 grams of KH2PO4and 5.45 grams of Dipotassium hydrogen phosphate was weighed, dissolved and diluted to 1000ml water and adjusted pH to 3 with orthophosphoric acid. Sonicated and filtered.

Mobile phase:
Mix a mixture of above buffer 300 ml (30%) and 700 ml of Acetonitrile (HPLC grade-70%) and sonicated and degassed with0.22µm filter paper. The mobile phase was pumped through the column at a flow rate of 1.0 ml/min.
SAMPLE AND STANDARD PREPARATION FOR THEANALYSIS
10 mg of imipenem and 10mg of cilastatin standards was transferred into 10 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 1.5 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase [7]. (The Concentration is 150 ppm &150 ppm of imipenem and cilastatin respectively.)

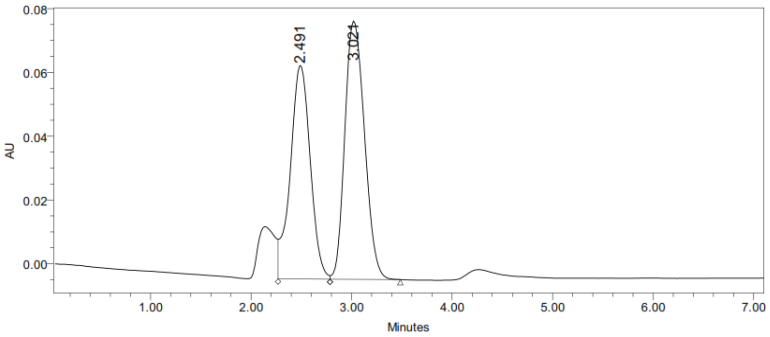
RESULT AND DISCUSSION:

Table-1: Trials for method development

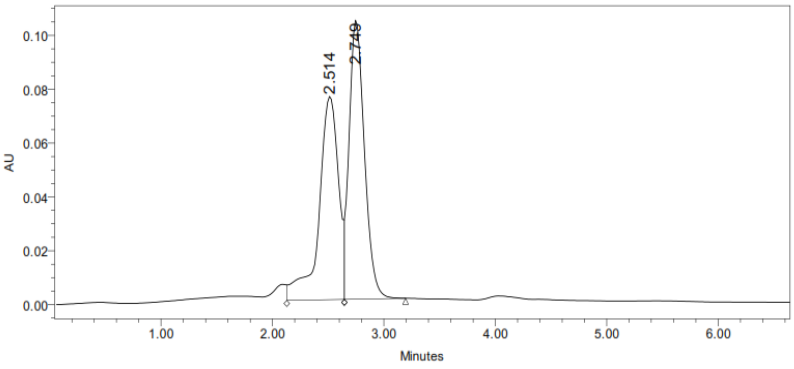
Column Used	Mobile Phase	Flow Rate(ml/min)	Wavelength	Observation	Result
Symmetry C18, 4.6x150mm,5µm i.d.	Methanol: water (50 :50%)	1.0	236nm	Both the Peaks was merged	Method rejected
Zodiac sil C18, 150mm x 4.6mm 5µm i.d.	Acetonitrile: Water (50 :50%)	1.0	240nm	Cilastatin peak only eluted	Method rejected
Hypersil RPC8, 4.5×150mm,5.0µmi.d.	ACN: pH 6.8 buffer (50 :50%)	1.0	240nm	Resolution was not good between the peaks	Method rejected
X-TerraC18, 4.6×250mm 5µm i.d.	ACN: pH 6.8 buffer (35 :65%)	1.0	238nm	Separation & peak shape was good but need to reduce run time	Method rejected
X-Terra C18 4.5×250mm 5µm i.d.	ACN: pH 3.0 buffer (50:50%)	1.0	238nm	Separation and peak shape were good.	Method Accepted



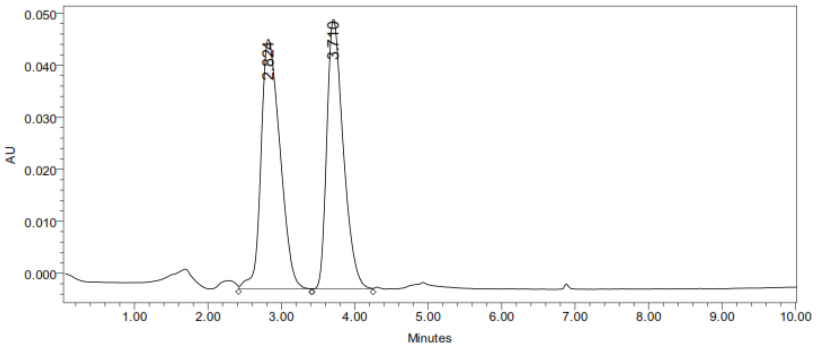
Trial-1



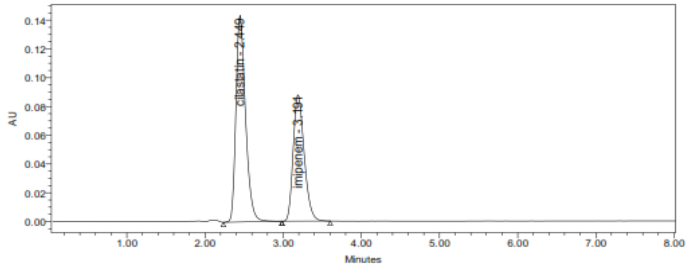
Trial-2



Trial-3



Trial-4



Trial-5Table 2: Peak results

	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

METHOD VALIDATION:

Accuracy: ***Recovery study:*** To determine the accuracy of the projected technique, recovery studies were distributed by adding totally different amounts (50%, 100%, and 150%) of pure drug of Imipenem and cilastatin were taken and side to the pre-analyzed formulation of concentration 150µg/ml [8,9]. From that proportion recovery values were calculated.

Table-3: Accuracy Readings of Imipenem

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	119642	5	4.98	99.85%	
100%	161803	10	9.97	98.23%	99.12%
150%	147889	15	14.95	99.29%	

Table-4: Accuracy Readings of Cilastatin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	165201	0.5	0.97	99.35%	
100%	192132	1.0	1.02	99.21%	99.40%
150%	205489	1.5	1.496	99.66%	

Precision:

Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Imipenem and cilastatin (API) the percent relative standard deviations were calculated for Imipenem and cilastatin [10].

Table-5: Repeatability Results of Precision

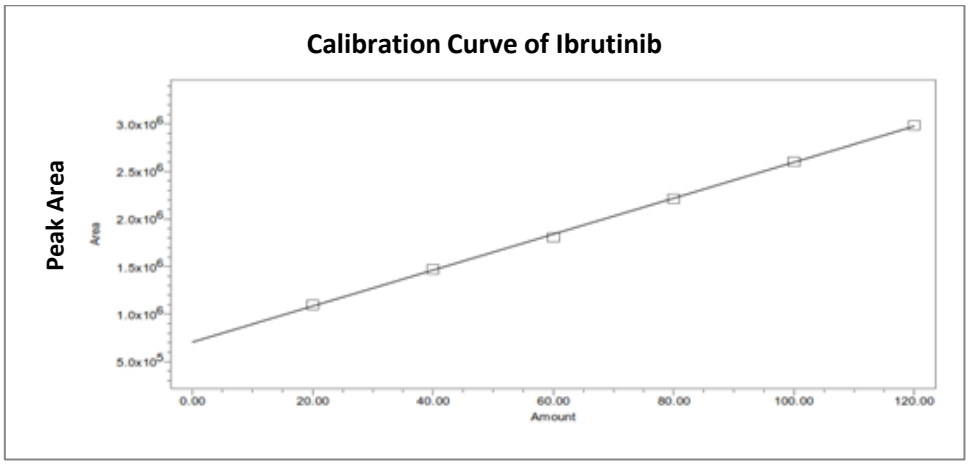
Replicates	Retention Time	Areas Imipenem	Areas Cilastatin
Replicate – 1	4.399	1197316	1600113
Replicate – 2	4.399	1171376	1600323
Replicate – 3	4.398	1207432	1620147
Replicate – 4	4.392	1212484	1605233
Replicate – 5	4.392	1203912	1670376
Replicate –6	4.393	1184991	1594239
Average		1196251.85	1615071.83
Standard Deviation		15452.53	28483.02
% RSD		1.291	1.764

Linearity and Range

The calibration curve showed good linearity in the range of 50-250µg/ml, for Imipenem and cilastatin 5-50µg/ml (API) with correlation coefficient (r^2) of 0.999.

Table-6: Linearity Results for IMIPENEM:

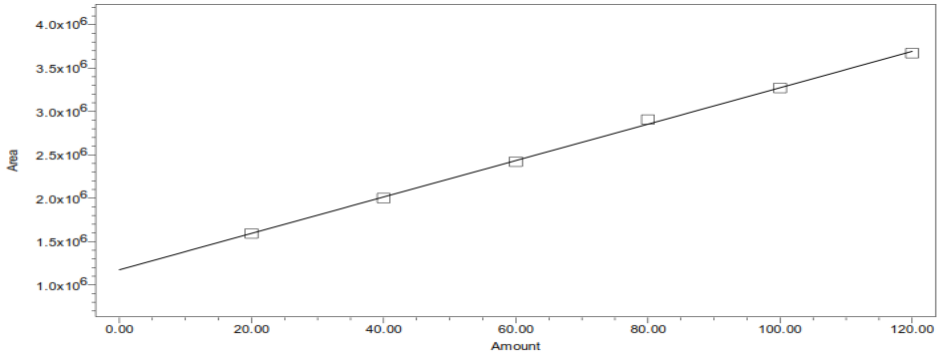
S.No	Linearity Level	Concentration	Area
1	I	50 ppm	242672.8
2	II	100 ppm	485412.5
3	III	150 ppm	729376
4	IV	200 ppm	969538
5	V	250 ppm	1214673
Correlation Coefficient			0.999



Calibration curve of Imipenem (API)

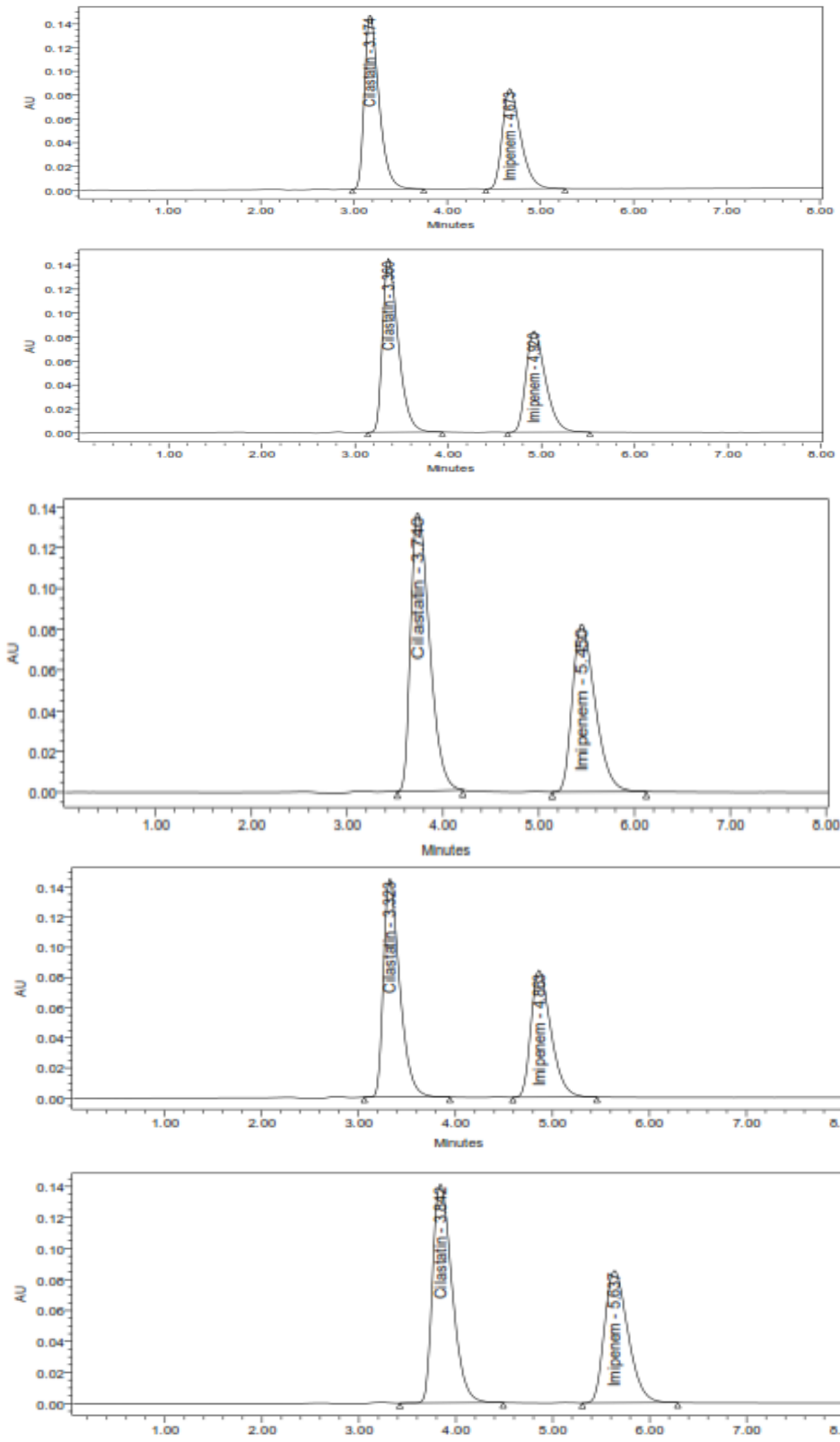
Table-7: Linearity Results for CILASTATIN

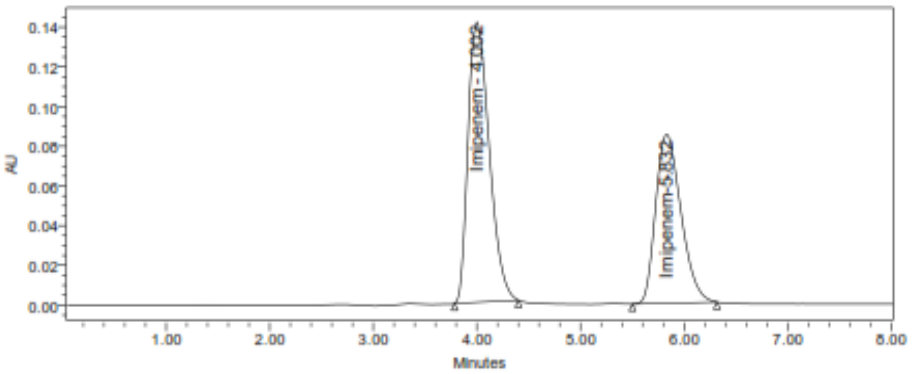
S.No	Linearity Level	Concentration	Area
1	I	5ppm	24373
2	II	10 ppm	48735
3	III	15ppm	73101
4	IV	20ppm	97490
5	V	25ppm	121851
Correlation Coefficient			0.999



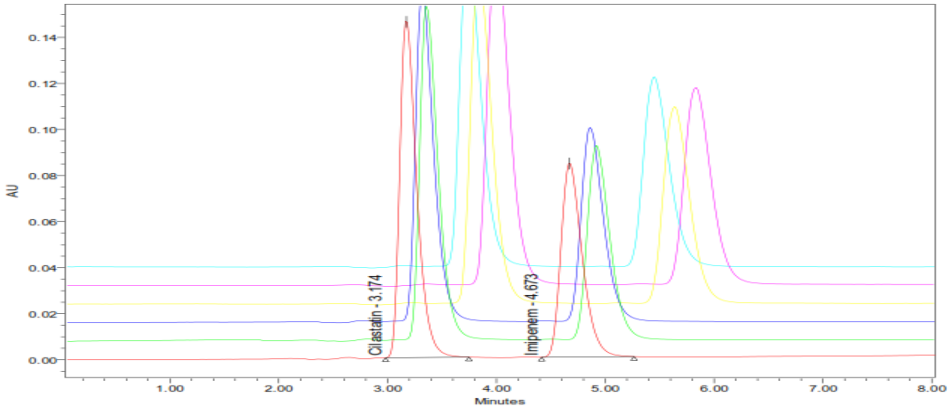
Showing calibration graph for cilastatin

Fig 4: Chromatograms showing linearity level-1 to level 5 (50ppm-250 ppm of IMIPENEM AND 5ppm - 50ppmof CILASTATIN) injection


Ciastatin - 3.174
Imipenem - 4.673
Ciastatin - 3.366
Imipenem - 4.926
Ciastatin - 3.745
Imipenem - 5.350
Ciastatin - 3.324
Imipenem - 4.865
Ciastatin - 3.842
Imipenem - 5.537
AU
Minutes



OVERLAY DIAGRAM



The linearity range of 50ppm-250ppm and 5ppm-50ppm of Imipenem and cilastatin and the correlation coefficient was found to be 0.999 and 0.999. (NLT 0.999).

LOD & LOQ: The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to respectively.

Detection limit

Fig 5: Chromatogram Showing Lod

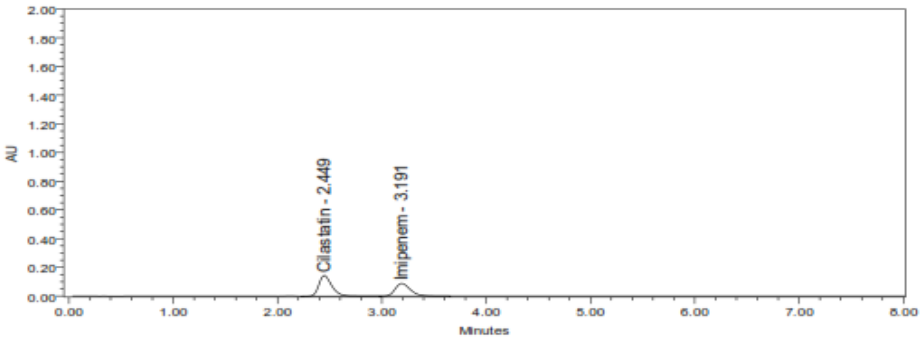


Table-8: Showing results for Limit of Detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Imipenem	371971	563584.141	2.14
Cilastatin	6413	480950612.3	0.0316

The LOD for Imipenem and cilastatin was found to be 2.14and 0.0316 respectively.

Quantitation limit

Fig 6: Chromatogram Showing LOQ

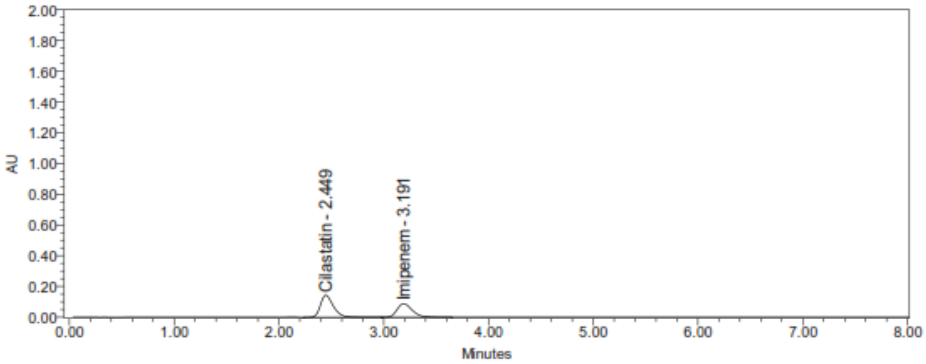


Table-9: Showing results for Limit of Quantitation

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Imipenem	371827.90	563365963	6.42
Cilastatin	5401.60	479884400	0.0948

System Suitability Parameter

System quality testing is Associate in nursing integral a part of several analytical procedures. The tests area unit supported the construct that the instrumentation, physics, Associate in Nursingalytical operations and samples to be analyzed represent an integral system that may be evaluated intrinsically [14]. Following system quality take a look at parameters were established.

Table-10: Dataof System Suitability Parameter

S.No.	Parameter	Limit	Result
1	Resolution	Rs > 2	Cilastatin and Imipenem: 4.3
2	% RSD	NMT≤ 2	Imipenem: 1.9 Cilastatin: 1.1
3	Theoretical plate	N > 2000	Imipenem: 2506 Cilastatin: 2596
4	Tailing Factor	T<2	Imipenem: 1.32 Cilastatin: 1.43

CONCLUSION

A sensitive& selective RP-HPLC method has been developed & validated for the analysis ofImipenem and cilastatin.

Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. The result shows the developed method is yet another suitable method for assay, purity & stability which can help in the analysis of Imipenem and cilastatin in different formulations.

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