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A Novel Method for the Simultaneous Estimation of Imipenem and Cilastatin by Using RP-HPLC in its Bulk and Pharmaceutical Dosage Form

Satheesh thotla*, R. Vasanthi, Dr. Alagar Raja.M, K.N.V Rao, Devid Banji

Departmernt of Pharmaceutical Analysis, Nalanda College of Pharmacy, Nalgonda, Telangana, India

A B S T R A C T

A new method was established for simultaneous estimation of Imipenem and cilastatin by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Imipenem and cilastatin by using Symmetry C18 column (4.6×150mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) Acetonitrile : phosphate buffer ($KH_2PO_4$and K_2HPO_4) pH 3 (pH was adjusted with ortho-phosphoric acid), detection wavelength was 240nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.449 mins and 3.191 mins. The % purity of Imipenem and cilastatin was found to be 100.27% and 99.87% respectively. The system suitability parameters for Imipenem and cilastatin such as theoretical plates and tailing factor were found to be 2438, 1.26 and 2235 and 1.33, the resolution was found to be 8.67. The linearity study of Imipenem and cilastatin was 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 was found to be 99.56% and 99.48%.

Keywords: Imepenam, Cilastatin, HPLC, Validation studies

A R T I C L E I N F O

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***Corresponding Author**
Satheesh Thotla
Departmernt of Pharmaceutical Analysis, Nalanda College of Pharmacy
Nalgonda, Telangana, India
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1. 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 bateria is attained by binding to pencillin binding proteins (PBPs). In E. coli and selected strains of P. aeruginosa, imipenem has shown to have the highest affinity to PBP-2, PBP-1a, and PBP-1b. This preferential binding to PBP-2 and PBP-1b results in the direct conversion of the individual cell to a spheroblast, which leads to rapid cell lysis and death without filament formation. chemically known as (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.

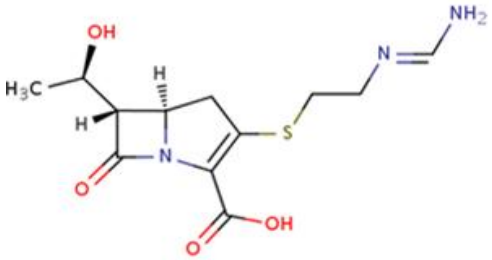

Figure 1: Imepenam

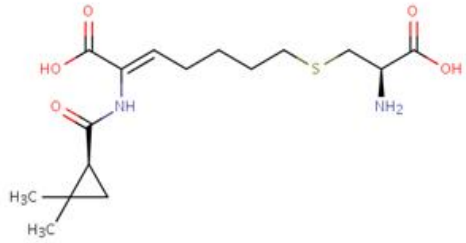

Figure 2: Cilastatin

Its Anti bacterial agent for the treatment of bacterial infections caused by susceptible and then second drug Cilastatin is a specific and reversible renal dehydro peptidase-I inhibitor. Since the antibiotic, imipenem, is hydrolyzed 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. Chemically Known as (2Z)-7-{[(2R)-2-amino-2-carboxyethyl] sulfanyl}-2-{[(1S)-2, 2-dimethylcyclopropyl] formamido} hept-enoincacid. Cilastatin is a competitive, reversible and specific inhibitor of dehydropeptidase-I enzyme.

2. Materials and Methods

Buffer preparation:

Preparation of Phosphate buffer :(pH: 3.0):

2.95 grams of $KH_2PO_4$and 5.45 grams of K_2HPO_4 was weighed and taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water and pH was adjusted to 3 with ortho phosphoric acid. The resulting solution was sonicated and filtered.

Standard Preparation:

10 mg Imipenem and 10 mg 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 1 ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Method development & Optimization:

Using Mobile phase consisting of different buffers and methanol at different concentrations and different mobile phase's pH values are attempted .The peak was observed that the shape and retention time of Imepenam and Cilastatin were found to be broad compared to the mixed phosphate buffer and acetonitrile composition of mobile phase. After selecting the best conditions based on peak performance, ACN: Phosphate buffer pH 3.0 (70: 30 % v/v) and HPLC using column is : zodiac sil RP C18 4.6×250mm 3.0µm , the run times of the proposed method was 10 mins with isocratic solution. Column temperature is 25°C,flow rate is 1ml/min, PDA Detector is mainly used this purpose ,after inject the standard solution volume was found to be 10µL. Retention times found were about 2.365 & 3.907 mins respectively.

Method validation:

Imepenam and Cilastatin standards taken to the 25mg was accurately weighed and transferred into a 25ml of volumetric flask containing HPLC grade Methanol s diluents. It was sonicated, dissolves completely and made volume up to the mark with the same solvent. The method was validated in accordance with ICH guidelines. The parameters assessed were precision, accuracy, linearity, specificity, robustness.

Table 1: Details of Instrument

S.No	Instrument	Model No.	Software	Manufacturer's name
1	HPLC Alliance	Waters 2695	Empower	Waters
2	PDA Detector	Waters 996	UV Win 5	Lab India
3	UV double beam spectrophotometer	UV 3000	-	Satorius
4	Digital weighing balance	BSA224SCW	-	Lab India
5	pH meter	AD102U	-	-
6	Ultra sonicator	SE60US	-	-

3. Results and Discussion

A new method was established for simultaneous estimation of Imipenem and cilastatin by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Imipenem and cilastatin by using SYMMETRY C18 column (4.6×150mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) Acetonitrile: phosphate buffer ($KH_2PO_4$and K_2HPO_4) pH 3 (pH was adjusted with ortho phosphoric acid), detection wavelength was 240nm.

The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.449 mins and 3.191 mins. The % purity of Imipenem and cilastatin was found to be 100.27% and 99.87% respectively. The system suitability parameters for Imipenem and cilastatin such as theoretical plates and tailing factor were found to be 2438, 1.26 and 2235 and 1.33, the resolution was found to be 8.67. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Imipenem and cilastatin was 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 was found to be 99.56% and 99.48%, %RSD for repeatability was 1.2 and 2.0, % RSD for intermediate precision was 1.1 and 1.1 respectively. LOD value was 2.17 and 6.60, and LOQ value was 0.032 and 0.1125 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Imipenem and cilastatinin API and Pharmaceutical dosage form.

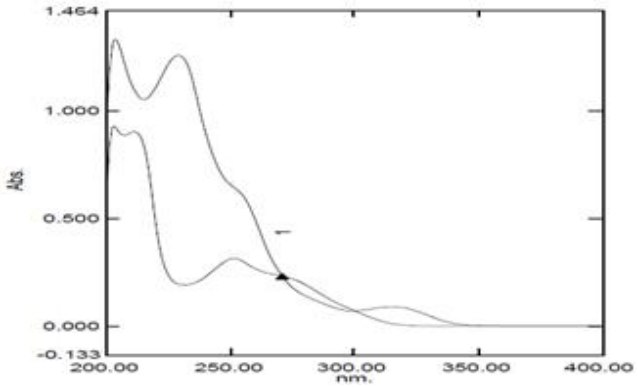

Figure 3: UV Specrum (Isobestic Point)

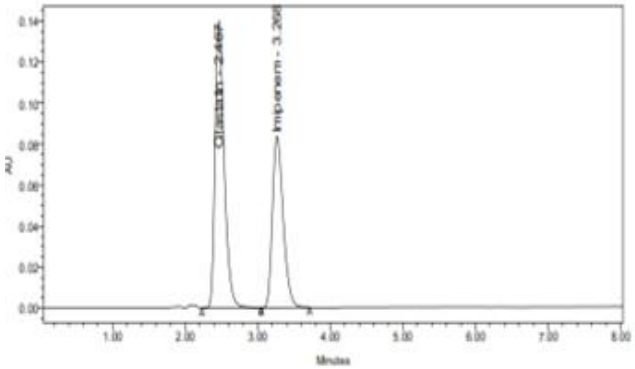

Figure 4

Table 1

	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

System suitability parameters

Method Validation:
Precision:

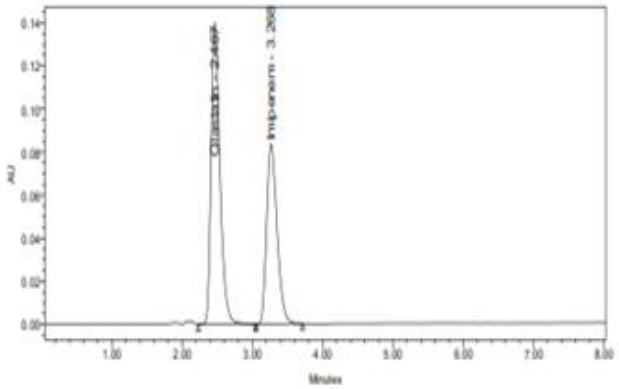

Figure 5

Table 2: 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					

Linearity

Table 3: 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
Correlation Coefficient			0.999

Acceptance Criteria: Correlation coefficient should be not less than 0.999

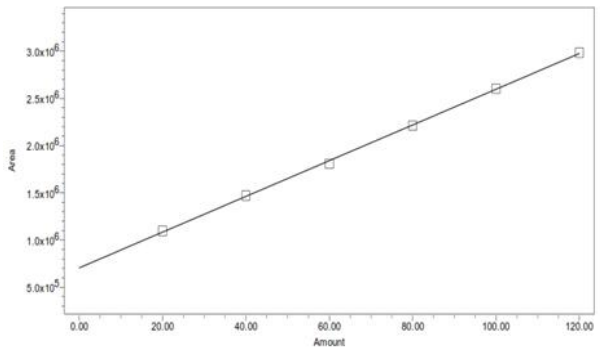

Figure 6: Showing calibration graph for Imipenem

Table 4: Linearity Results for Cilastatin

S.No	Linearity Level	Concentration	Area
1	I	5ppm	56472
2	II	10 ppm	73841
3	III	15ppm	92655
Correlation Coefficient			0.999

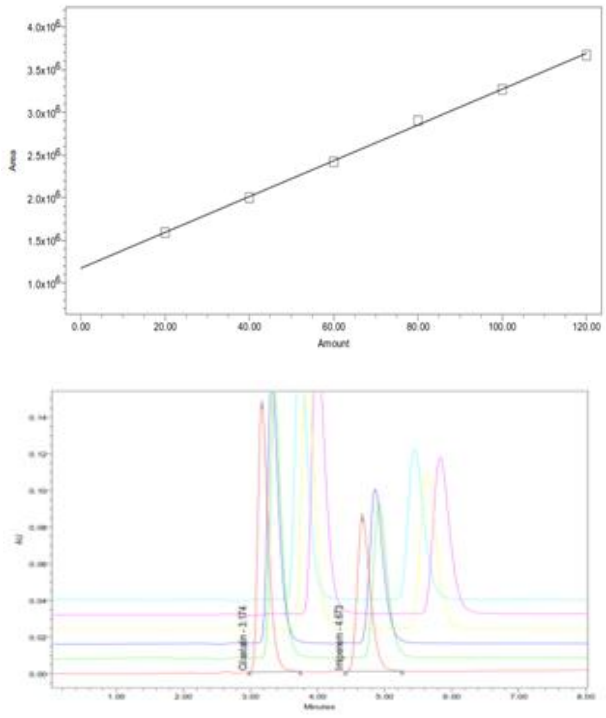

Figure 7: Showing calibration graph for cilastatin

Overlay Report

The linearity study was performed for concentration 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).

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%. .

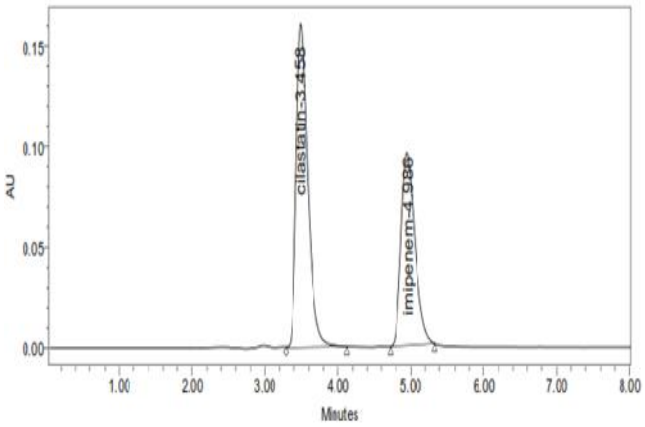

Figure 8: Chromatogram showing less flow rate 0.8ml/min

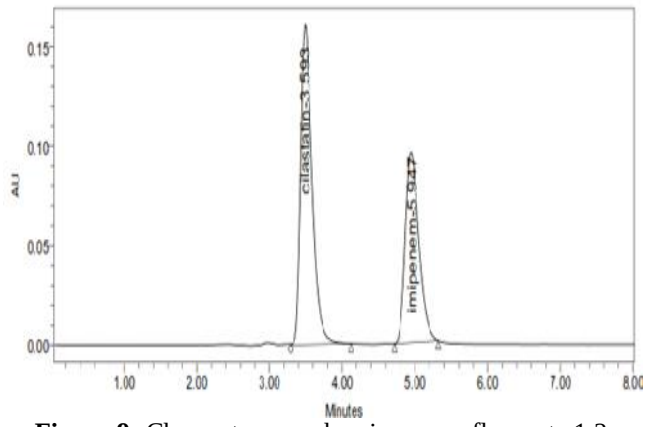

Figure 9: Chromatogram showing more flow rate 1.2 ml/min

Table 5: Showing system suitability results for Imipenem

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

Table 6: Showing system suitability results for Cilastatin

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

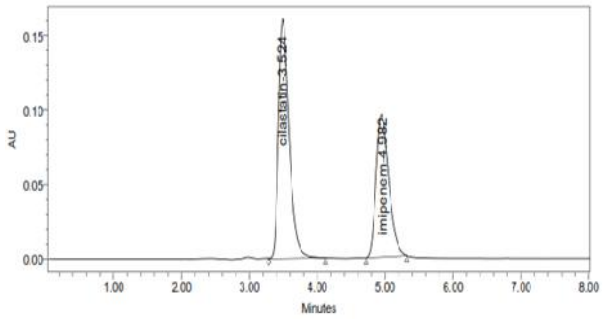

Figure 10: Chromatogram showing more organic phase ratio

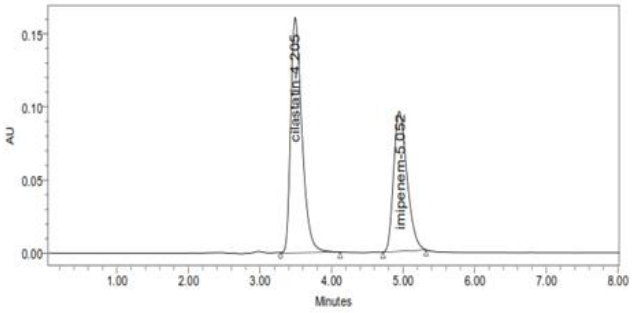

Figure 11: Chromatogram showing less organic phase ratio

Table 7: 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	2347	1.44
2	*Actual	2294	1.27
3	5 % more	2239	1.13

Table 8: 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	5437	0.99
2	*Actual	4891	1.03
3	5 % more	4817	1.05

4. Conclusion

Hence it can be concluded that the proposed HPLC method is sensitive and reproducible for the determination of related substances in Imepenam and Cilastatin. The major advantage of this method was short retention time.

Table 9: Showing accuracy results for Imipenem

%Concentration (at specification level)	Average area	Amount Added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	1193706	5	4.96	99.91%	99.56%
100%	1601711	10	9.98	99.18%	
150%	1479921	15	15.02	99.60%	

Table 10: Showing accuracy results for Cilastatin

%Concentration (at specification level)	Average area	Amount Added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	1656163	0.5	0.99	99.53%	99.47%
100%	1920586	1.0	1.05	99.38%	
150%	2058167	1.5	1.495	99.52%	

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