WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.63
Volume 15, Issue 11, 2535-2545. Review Article ISSN 2277–7105

METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF IMIPENEM AND CILASTATIN SODIUM POWDER FOR INJECTION BY RP-HPLC

Dr. Ch. Saibabu*[1], M. Praveen Kumar[2], Dr. M. Bhaskar[3]

[1]*Professor, Department of Pharmaceutics, Malineni Lakshmaiah College of Pharmacy, Singarayakonda, Prakasam-523101, Andhrapradesh, India.

[2]Department of Pharmacy, Malineni Lakshmaiah College of Pharmacy, Singarayakonda, Prakasam-523101, Andhrapradesh, India.

[3]Professor, Department of Pharmaceutical Analysis, Malineni Lakshmaiah College of Pharmacy, Singarayakonda, Prakasam-523101, Andhrapradesh, India.

Article Received on 05 May 2026,
Article Revised on 25 May 2026,
Article Published on 03 June 2026,

https://doi.org/10.5281/zenodo.20537012

***Corresponding Author**

Dr. Ch. Saibabu

Professor, Department of Pharmaceutics, Malineni Lakshmaiah College of Pharmacy, Singarayakonda, Prakasam-523101, Andhrapradesh, India.

How to cite this Article: Dr. Ch. Saibabu*[1], J. Praveena[2], Dr. M. Bhaskar[3] (2026). Method Development And Validation For Simultaneous Estimation Of Imipenem And Cilastatin Sodium Powder For Injection By Rp-Hplc. World Journal of Pharmaceutical Research, 15(11), 2535-2545.

ABSTRACT

A reserve phase liquid chromatography (RP-HPLC) method has been developed and subsequently validated for the determination of Imipenem and Cilastatin sodium bulk and its pharmaceutical formulation. Separation was achieved with a Inertsil ODS SP C_{18},(particles with 5µm), 250mm x 4.6mm column and Sodium 1-hexane sulfonate and potassium dihydrogen orthophosphate buffer pH 6.8 as mobile phase at a flow rate of 2.5 ml/min and the column temperature was maintained at 50˚c.Dual wavelength detector was performed at 254 nm and sample temperature was maintained at 5˚c with a run time of 20 minute. The method rapid simple and sensitive. The described method of Imipenem and Cilastatin is linear over a range of 60µg/ml and 120µg/ml with correlation coefficient of 0.998, 0.998 respectively for both Imipenem and Cilastatin. The method precision for determination of assay was below 2.0% RSD. The method enables accurate, precise and rapid analysis of Imipenem and Cilastatin. It can be conveniently adopted for routine quality control analysis of bulk and pharmaceutical formulations.

KEYWORDS: Imipenem and Cilastatin, HPLC, Assay, Method development.

INTRODUCTION

Imipenem (IPM) is official in the United States Pharmacopoeia[9] and the European Pharmacopoeia.[10] United States Pharmacopoeia describes a high performance liquid chromatographic method for the assay of IPM in pharmaceutical dosage forms. Visible spectrophotometric methods using brucine/sodium periodate,[11] bromosuccinimide/celestin blue,[12] haematoxylin/chloramine–T[13] and potassium ferricyanide/ferric chloride[14] as chromogenic reagents were proposed by Babu et al., for the quantification of IPM in bulk and injection formulations.

Garcia- Capdevila et al.,[15] and Walter et al.,[16] developed HPLC with UV detection methods for the determination of IPM concentrations in human plasma. These two methods are applied to pharmacokinetic studies in patients. HPLC-UV detector methods proposed by Dehghanzadeh et al.,[17] and Dong et al.,[18] were applied to determine the concentration of IPM in hospital sewage samples and sputum samples, respectively. Babu et al.,[12] and Taniguchi et al.,[19] reported HPLC with UV detection method and capillary zone electrophoresis method for the quantification of IPM in bulk and injection forms. Two reports of IPM quantification in human urine sample by voltammetry were found in the literature.[20,21] Regarding the determination of Cilastatin (CSN) alone, only one method is found in the literature. The reported one is a HPLC with UV method and applied for its quantification in human plasma & urine samples.[22] El-Kosasy et al.,[23] Forsyth & Ip,[24] Parra et al.,[25] Baldha et al.,[26] and Omar & Itab[27] described derivative UV spectrophotometric methods for the simultaneous quantification of IMP and CSN.

All the reported UV spectrophotometric methods[24-27] are applied for the bulk and injection forms, except the El-Kosasy et al.[23] method which is applied for human urine sample. HPLC methods were described by Sandhya rani et al.,[28] Srinivasan et al.[29] Natalija et al.[30] for the simultaneous determination of IPM and CSN powder in injection dosage forms. A hydrophilic interaction chromatography/mass spectrometry assay for IPM and CSN was reported by Zhe-Yi et al.[31] and Xu et al.[32] Zhe-Yi et al.[31] method is used for the measurement of IPM and CSN in human plasma, whereas Xu et al.[32] method is applied for the determination of IPM and CSN in rat plasma, monkey plasma, and mouse blood.

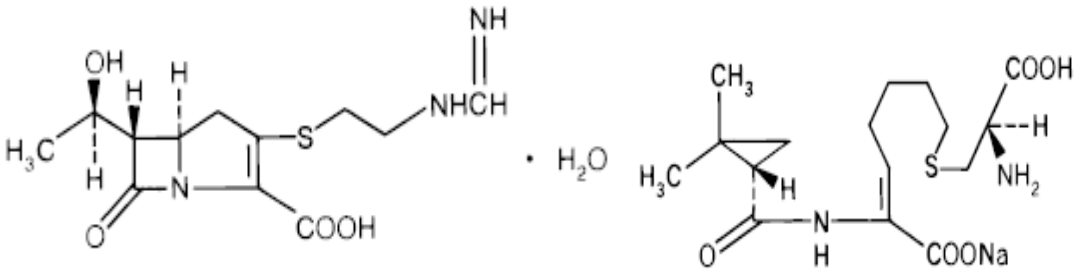

Figure 1: Chemical structure of imipenem and cilastatin sodium.

MATERIALS AND METHODS

Instrumentation

Shimadzu HPLC class LC series equipped with two LC-10 AT, VP pumps and variable wavelength programmable UV detector, memmert type hot air oven, model BTI30, Bio Technics India (Mumbai, India), Scimadzu electronic weighing balance (Kyoto, Japan) TX423L model, and Inertsil -ODS C18, (250 mm × 4.6; 5 µm particle size) column were used for present study.

Materials, Chemicals and solvents

For the present investigation the reference standards of IPM and CSN are obtained from the Hetero pharmaceutical Ltd, Hyderabad, India as gift sample and Cilanem 500 mg injection formulation (Ranbaxy Laboratories Ltd., India) labeled to contain 500 mg of IPM and 500 mg of CSN per one vial was purchased from the local medical store as well as HPLC grade methanol and acetonitrile was purchased from Merck (India) Ltd., Mumbai and analytical reagent grade hydrochloric acid, sodium hydroxide and hydrogen peroxide were from Sdfine Chem limited (Mumbai, India). Mille Q water was used throughout the process.

Table No. 1: Chromatographic condition.

Parameter/ conditions	**Description/Values**
Column used	Inertsil ODS SP C_{18}, particles with 5µm, 250mm x 4.6mm
Buffer used	0.54g of potassium dihydrogen orthophosphate and adjust pH to 6.8±0.05 with 0.5Nsodiumhydroxide or 0.5M orthophosphoric acid
Mobile phase	Sodium 1-hexane sulfonate + 0.54g of potassium dihydrogen orthophosphate and adjust pH to 6.8±0.05 with 0.5Nsodiumhydroxide or 0.5M orthophosphoric acid.
Injection volume	10µL
Elution mode	Isocratic
Flow rate	2.5ml/min
Run time	20min

Sample compartment temperature	5°C
Column temperature	50°C
Wavelength & detector	254nm&dual wavelength detector

0.5N sodium hydroxide solution: Dissolve 2.0 g of sodium hydroxide in water to produce 100ml.

Preparation of buffer: Weigh and dissolve about 0.54 g of potassium dihydrogen orthophosphate in 3600ml of water adjust the pH to 6.8 ± 0.05 with 0.5N sodium hydroxide solution 0r 0.5m ortho phosphoric acid solution and dilute to 4000ml with water. Filter the solution through a 0.2 µm membrane filter and degas.

Preparation of mobile phase: Weigh and dissolve about 2.0 g of sodium 1-hexane sulfonate in 800ml of buffer solution adjust the pH to 6.8± 0.05 with 0.5n sodium hydroxide solution or 0.5M orthophosphoric acid solution and dilute to 1000ml buffer solution , Filter the solution through a 0.2 µm membrane filter and degas.

Preparation of saline TS solution: Weigh and dilute about 0.9g of sodium chloride in 100ml water.

Preparation of 0.1% W/V sodium bicarbonate solution: Dissolve about 0.1g of sodium bicarbonate in 100ml water.

Preparation of standard stock solution

1. Weigh accurately and transfer about equivalent 50mg of Imipenem monohydrate working standard and Equivalent 50mg of Cilastatin ammonium salt working standard into a 100 ml volumetric flask.
2. Add 20 ml of saline TS solution 2.0 ml of a 0.1% W/V sodium bicarbonate solution and 60 ml of buffer solution dissolved by shaking and sonication, dilute for volume with buffer solution and mix to homogenize.
3. Filter through 0.45 µm membrane filter.

Preparation standard test solution: Above solution take 1 ml into 50 ml volumetric flask dilute with buffer solution.

Preparation of sample stock solution

1. Weigh accurately and transfer about 108 mg of Imipenem and Cilastatin sodium powder for injection into a 100ml volumetric flask. Add 20 ml of saline TS solution and 60 ml of buffer solution dissolve by shaking and sonication and dilute to volume with buffer solution Mix and homogenize.
2. Filter through 0.45 µm membrane filter.

Preparation of sample test solution: Above solution take 1 ml into 50 ml volumetric flask dilute with buffer solution.

Evaluation of System suitability: Inject 10 µl of standard preparation six replicate injections into the chromatograph using the above chromatographic parameters .measure the peak area response for the analyte peak and evaluate the system suitability parameters as directed.

Acceptance criteria

ASSAY: Separately inject (about 10 µl) of blank , standard ,sample preparation in to the chromatograph and record the chromatograms and measure the peak area responses for the analyte peaks calculate the % content of Imipenem and Cilastatin sodium in the combined portion of Imipenem and Cilastatin sodium powder for injection (500mg/500mg) by using formula.

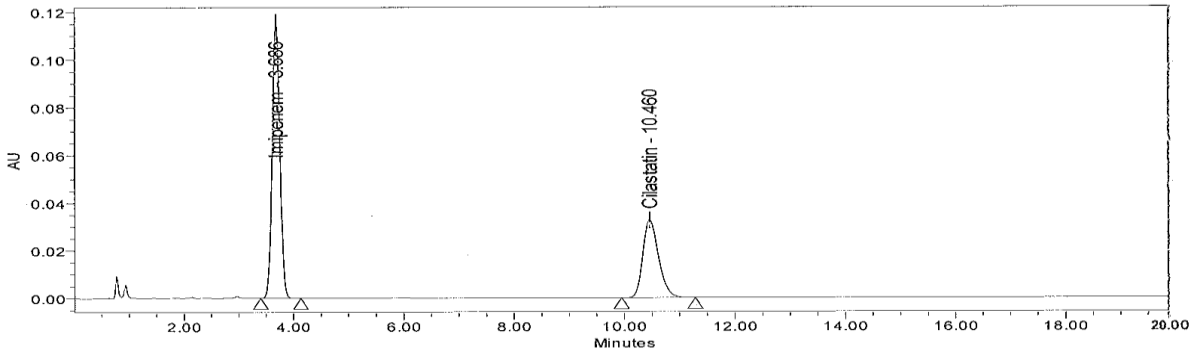

Figure No. 2: Assay of Imipenem and Cilastatin sodium powder chromatogram.

Specificity: The specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak .the study was performed by injecting blank, Standard solution and sample solution were injected into the chromatographic system. Retention times obtained from standard and sample were compared for identification of analyte.

Precision: The precision of the analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. The precision of analytical method is expressed as the standard deviation or relative standard deviation of series of measurements.

Table No. 2: Comparison of method precision and intermediate precision.

	Imipenem & Cilastatin		
	Assay in %		
	Set. No.	Imipenem	Cilastatin
Method precision	1	96.801	94.299
	2	96.548	94.139
	3	97.601	94.460
	4	97.147	94.290
	5	98.492	96.474
	6	96.546	94.202
Intermediate precision	1	97.601	94.460
	2	97.147	94.290
	3	96.436	95.824
	4	98.576	96.424
	5	96.472	95.202
	6	96.801	95.800
Mean of 12 determinations		97.180	95.00
%RSD of 12 determination		0.8	0.9

System precision: Six injections were prepared individually Imipenem monohydrate and Cilastatin ammonium salt working standards as per test method and injected each solution. The peak responses were recorded. The % RSD for Imipenem and Cilastatin peak area of standard preparation was calculated.

Method precision: In method precision, a homogeneous sample of a single batch should be analyzed six times. This indicates whether a method is giving consistent results for a single batch. The method precision was performed.

Inter mediate precision / ruggedness: The intermediate precision as been carried out to ensure that the analytical results will remain unaffected with change in instrument, analyst, column and day. The method precision set was repeated by different analyst on different instrument using different lot on column on different day.

Linearity: A Series of solutions are prepared using Imipenem monohydrate and Cilastatin ammonium salt working standard at concentration levels from 60µg to 140µg of target

concentration. Measure the peak area response of solution and constructed a plot between concentrations vs. peak area and calculate correlation co-efficient and regression co-efficient.

Table No. 3: Result of linearity of Imipenem.

S. No.	Concentration(µg/ml)	Peak area
1	0	0
2	60	660431
3	80	864618
4	100	1140316
5	120	1352168
6	140	1578642
	Slope	11224
	Regression coefficient	0.998

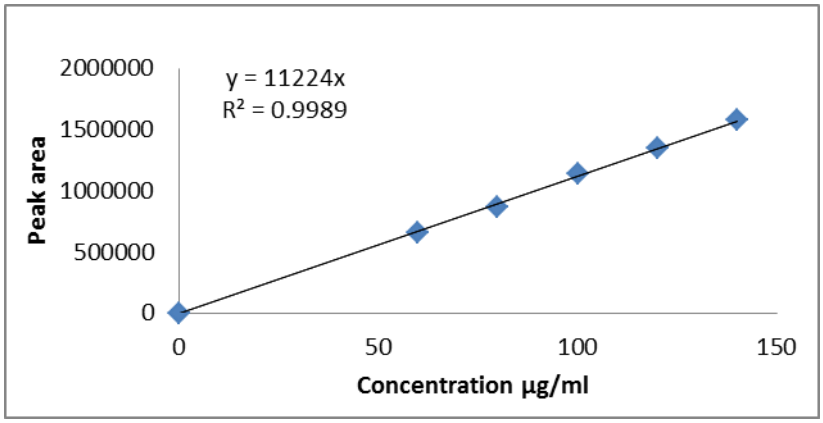

Figure No. 3: linearity of Imipenem.

Table No. 4: Result of linearity of Cilastatin.

S. No.	Conc.(µg/ml)	Peak area
1	0	0
2	60	381143
3	80	528524
4	100	689645
5	120	812148
6	140	939642
	Slope	6724
	Regression coefficient	0.998

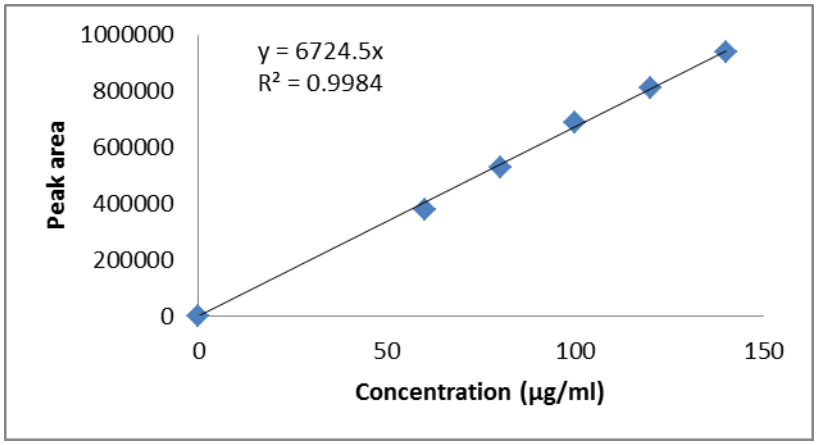

Figure No. 4: Linearity of Cilastatin

Accuracy: The accuracy of analytical method is the close of test results obtained by that method to be the true value the accuracy of analytical method should be established across its range. Spike known quantity of Imipenem and Cilastatin standards at 80%, 100% and 120% level. This sample was analyzed in to triplicate for each level. From the result % recovery was calculated.

Table No. 5: Accuracy preparation.

S.No.	%Level	Amount of Imipenem added (in mg)	Amount of Cilastatin added (in mg)	Total volume (in ml)
1	80	50	50	100
2	80	50	50	100
3	80	50	50	100
4	100	50	50	100
5	100	50	50	100
6	100	50	50	100
7	120	50	50	100
8	120	50	50	100
9	120	50	50	100

Robustness: It is an analytical method which explain the final values remain unchanged even there is a drastic changes in the method.As part of evaluation of robustness, deliberate change in the flow rate, mobile phase composition and temperature was made to evaluate the impact on the method.

LOD and LOQ: According to USP "LOD is the lowest concentration of analyte that can be detected, but necessarily not quantities, under the stated experimental conditions.

According to USP "LOQ is the lowest concentration in a sample that may be measured with an acceptable level of accuracy and precision, under stated experimental conditions."

SUMMARY AND CONCLUSION

The scope and objective of the present work is to optimize the chromatographic conditions to develop RP-HPLC method for the simultaneous estimation of Imipenem and Cilastatin sodium powder for injection dosage form and same is validated. RP-HPLC method generate large amount of quality data which serve as highly powerful and convenient analytical tool.

Literature review indicates that HPLC and UV-Spectrophotometric individual and combined methods have been reported for Imipenem and Cilastatin. The scope and objective of the present work is to optimize condition to develop and validate the simultaneous estimation of Imipenem and Cilastatin sodium by RP-HPLC.

Based on literature review, a RP-HPLC method was developed on Inertsil ODS SP C_{18}, particles with 5µm, 250mm x 4.6mm column with potassium dihyrogen orthophosphate buffer (pH 6.8±0.05) with 0.5N sodium hydroxide or 0.5M orthophosphoric acid and sodium 1-hexane sulfonate(100%) as mobile phase at a flow rate of 1.0 ml/min with UV detection at 254 nm for estimation of Imipenem and Cilastatin sodium . The run time of the RP-HPLC procedure is only 20 minutes. The Proposed RP-HPLC method was suitable technique for estimation of Imipenem and Cilastatin sodium powder for injection in pharmaceutical dosage form without any interference from other excipients.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to Azidus Laboratories, Chennai, India, for their valuable support in providing a literature survey and facilitating this research.

CONFLICT OF INTEREST: The authors declare that they have no conflicts of interest related to this research.

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