

## STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF IMPENEM AND CILASTATIN IN INJECTION FORMULATIONS

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### ABSTRACT

Reverse Phase High Performance Liquid Chromatographic forced degradation method have been developed and validated for the simultaneous estimation of Imipenem (IPM) and Cilastatin (CSN) in Injection formulations. Both drugs and their degradation studies were conducted by using Inertsil- C18, BDS (250 mm x 4.6;5 $\mu$ m particle size) column and the separation was achieved by using methanol : acetonitrile (80:20) as mobile phase and flow rate was 1.0ml/min with runtime 8mins at ambient temperature, and the retention time for both drugs was 2.930 mins for IPM and 4.215 for CSN respectively. This method was linear at the concentration range of 20-80 $\mu$ g/ml ( $R^2=0.9990$  &  $R^2=0.9991$ ) for both IPM and CSN respectively. The

stability indicating studies of the method was developed by subjecting both drugs to various stress conditions like acid, base, oxidation, heat and photo light. There were no interfering peaks from excipients or degradation products have developed due to variable degradation conditions. Degradation products produced as a result of forced degradation studies did not interfere with the detection of Imipenem and Cilastatin and the method can thus be considered stability indicating.

**KEYWORDS:** Imipenem, Cilastatin, Stability indicating, Injection, RP-HPLC, Simultaneous, Forced degradation.

## INTRODUCTION

Imipenem (IPM) is official in the United States Pharmacopoeia<sup>[9]</sup> and the European Pharmacopoeia.<sup>[10]</sup> 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<sup>[11]</sup>, bromosuccinimide/celestine blue<sup>[12]</sup>, haematoxylin/chloramine-T<sup>[13]</sup> and potassium ferricyanide/ferric chloride<sup>[14]</sup> as chromogenic reagents were proposed by Babu *et al.*, for the quantification of IPM in bulk and injection formulations.

Garcia- Capdevila *et al.*,<sup>[15]</sup> and Walter *et al.*,<sup>[16]</sup> 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.*,<sup>[17]</sup> and Dong *et al.*,<sup>[18]</sup> were applied to determine the concentration of IPM in hospital sewage samples and sputum samples, respectively. Babu *et al.*,<sup>[12]</sup> and Taniguchi *et al.*,<sup>[19]</sup> 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.<sup>[20,21]</sup>

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.<sup>[22]</sup> El-Kosasy *et al.*<sup>[23]</sup>, Forsyth & Ip<sup>[24]</sup>, Parra *et al.*<sup>[25]</sup>, Baldha *et al.*<sup>[26]</sup> and Omar & Itab<sup>[27]</sup> described derivative UV spectrophotometric methods for the simultaneous quantification of IMP and CSN.

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

To the best of our knowledge, no stability indicating RP-HPLC method with UV detector was reported for the simultaneous estimation of the two drugs (IPM and CSN). The present investigation was aimed to develop and validate a sensitive, precise and accurate stability indicating RP-HPLC method with UV detector for the simultaneous quantification of IPM and CSN in bulk and combined injection formulation.

## 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.

### Mobile phase

The mobile phase used was methanol and acetonitrile in the ratio of 80:20 *v/v* in the present investigation.

### Preparation of solutions

The standard stock solution was prepared by dissolving 100 mg of IPM and 100 mg of CSN in 100 ml mobile phase. Working standard solutions equivalent to 20-80 µg/ml IPM and CSN was prepared from stock solution by appropriately diluting the stock standard solution with the mobile phase. The sample solution prepared as an accurately weighed amount equivalent to 100 mg of IPM and 100 mg of CSN was transferred into a 100 ml calibrated flask, and dissolved in about 40 ml of mobile phase. The contents of the flask were swirled, sonicated for 20 min, and completed to volume with the mobile phase. The contents were mixed well

and filtered through millipore membrane filter. The resultant filtrate was diluted appropriately with the mobile phase to get a final concentration of 40 µg/ml IPM and CSN for the analysis.

### **Chromatographic conditions**

Inertsil -ODS C18 (250 x 4.6 mm; 5 µm particle size) analytical column was used for separation and simultaneous analysis of IPM and CSN in the presence of the stress degradants. The column temperature was maintained at 27±1°C. The separation was carried out under isocratic elution. The flow rate was maintained as 1.0 ml/min. The injection volume was 20 µl. The eluents were detected at 248 nm. The total run time is 8 minutes.

## **METHOD DEVELOPMENT**

### **General analytical procedure**

Working standard solutions equivalent to the concentrations 20-80 µg/ml IPM and CSN were prepared by appropriate dilution of the stock standard solution with the mobile phase. 20µl aliquot of each solution was injected automatically into the column in triplicate. The mobile phase is pumped from the solvent reservoir to the column at a flow rate of 1 ml/min. The peaks were determined at 248 nm. The peak area of each concentration was plotted against the corresponding concentration to get the calibration graph and regression equation was derived.

### **Procedure for injection sample**

20µl of the sample solution prepared was injected into the HPLC system in triplicate. The general analytical procedure was applied. The area under the peak of each drug was noted. The IPM and CSN content in the injection dosage form were quantified using the corresponding calibration graph or corresponding regression equation.

### **Stress degradation studies**

Stress degradation studies were carried out using different ICH prescribed stress conditions such as acidic, basic, oxidative, thermal and photolytic stresses.<sup>[33]</sup>

### **Acid degradation**

Powder equivalent to 100 mg of IPM and CSN was taken in 100 ml volumetric flask. Five ml of 0.1N HCl was added to the flask and kept at 80°C reflux condition for 2 h. After completion of the stress, the solution was neutralized by using 0.1N NaOH and completed up to the mark with the mobile phase.

**Base degradation**

Powder equivalent to 100 mg of IPM and CSN was taken in 100 ml volumetric flask. Five ml of 0.1N NaOH was added in the flask and kept at 80 °C reflux condition for 2 h. After completion of the stress, the solution was neutralized by using 0.1N HCl and completed up to the mark with the mobile phase.

**Oxidative degradation**

Powder (equivalent to 100 mg of IPM; 100 mg of CSN) and 5 ml of 20% H<sub>2</sub>O<sub>2</sub> were added in 100 ml volumetric flask. The flask was kept at 80 °C reflux condition for 2 h. After completion of the stress, the 100 ml flask was completed up to the mark with the mobile phase.

**Thermal degradation**

For this, powder (equivalent to 100 mg of IPM; 100 mg of CSN) was taken in a glass petri dish and placed in hot air oven at 105°C for 2 h. After specified time, the tablet powder was transferred to a 100 ml volumetric flask and made up to the mark with the mobile phase.

**Photolytic degradation**

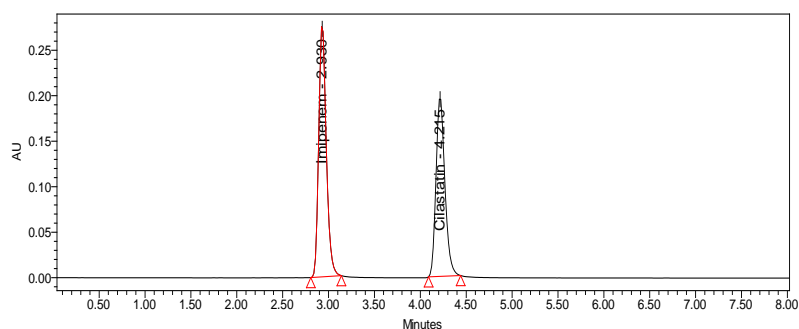
For photolytic degradation study, powder equivalent to 100 mg of IPM and CSN was transferred into a glass petri dish and placed in the direct sunlight for 2 h. After completion of the stress, the tablet powder was transferred to a 100 ml volumetric flask and made up to the mark with the mobile phase.

**RESULTS AND DISCUSSIONS****HPLC Parameters optimization**

The main aim of this study is to simultaneously analyze IPM and CSN in the presence of stress degradation products with sufficient resolution in reasonable analysis time. To obtain a good chromatographic condition, various mobile phases with various flow rates in isocratic elution mode were also tested to obtain good chromatographic condition:

1. Methanol: Water (90:10, v/v)
2. Acetonitrile: Water (90:10, v/v)
3. Acetonitrile: Methanol (80:20, v/v)
4. Methanol: Acetonitrile (80:20, v/v)

The peak shape and system suitability parameters of IPM and CSN were good while using Inertsil-ODS C18 (250 mm × 4.6 mm, 5 μm) column. Hence this analytical column was selected. The good performance and better separation were achieved with the mobile phase combination methanol and acetonitrile in the ratio of 80:20 v/v using Inertsil-ODS C18 (250 mm × 4.6 mm, 5 μm) column. The isocratic elution with a flow rate of 1 ml/min was optimized. Under the optimized chromatographic conditions, the chromatogram (Fig.1.) obtained, demonstrated a good separation of the IPM (2.930 min) and CSN (4.215 min) from each other.



**Fig. 1: Chromatogram of Imipenem and Cilastatin after method optimization.**

## METHOD VALIDATION

The optimized RP-HPLC method for simultaneous assay of IPM and CSN was validated according to ICH guidelines<sup>[34]</sup> with respect to system suitability, linearity, sensitivity, accuracy, precision, ruggedness, and robustness.

### System suitability

The system suitability test was evaluated from five replicate injections of the standard solution containing 40 μg/ml IPM and CSN. The two peaks were well resolved and the precision of injections for all the peaks was acceptable. The percent relative standard deviation of the IPM and CSN peak area responses were determined to be less than 1. The USP tailing factor and USP plate count were also calculated. The results of system suitability in comparison with the required limits are shown in Table.1 and are found to be within the accepted limits.

**Table No 1: System suitability of the HPLC method.**

Parameters	Value		Recommended limits
	Imipenem	Cilastatin	
Retention time	2.92546 (%RSD – 0.041)	4.213018 (%RSD – 0.026)	RSD $\leq$ 2
Peak area	677206.8 (%RSD – 1.071)	3680071 (%RSD – 0.167)	RSD $\leq$ 2
USP plate count	5395.809	6683.919	> 2000
USP tailing factor	1.485127	1.14032	$\leq$ 2
Resolution	-	5.36	> 3

**Linearity and range**

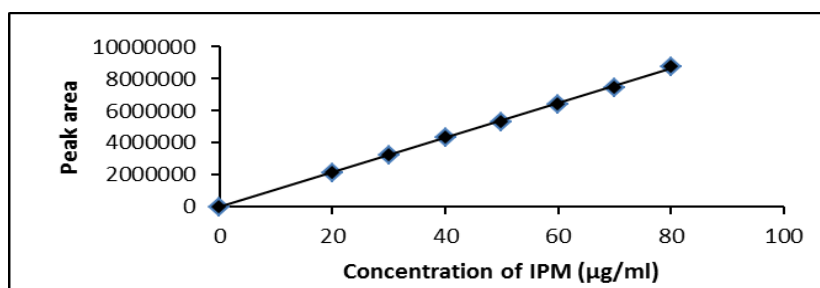
The linearity of the method was determined by analyzing seven different concentrations of each drug. The calibration curve was plotted by area under the peak responses of the drugs against their corresponding concentrations. Calibration curves were linear over the concentration range of 20-80  $\mu\text{g/ml}$  for IPM (Figure.2) and 20-80  $\mu\text{g/ml}$  for CSN (Figure.3.), respectively. The corresponding regression equation and regression coefficient are given in Table.2. The results demonstrate a good correlation between the peak areas of the drugs and their corresponding concentrations.

**Sensitivity**

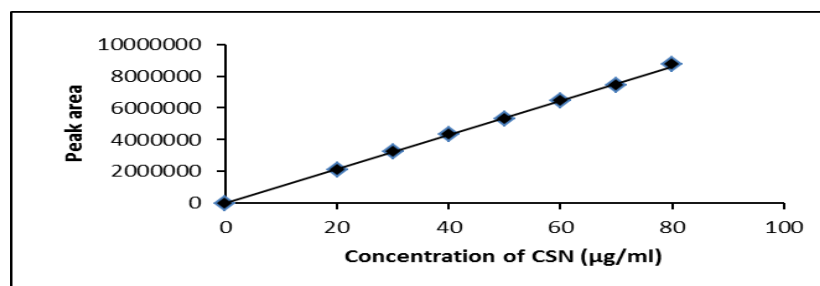
The sensitivity of the method was assessed by calculating limit of detection (LOD) and limit of quantification (LOQ) according to ICH guidelines.<sup>[34]</sup> The results are summarized in Table 3. The low values of LOD and LOQ demonstrate the sufficient sensitivity of the proposed method for the simultaneous estimation of the selected drug combination.

**Table No 2: Linearity and sensitivity of Imipenem and Cilastatin.**

Parameter	Imipenem	Cilastatin
Linearity ( $\mu\text{g/ml}$ )	20-80	20-80
Regression equation ( $y^* = m x^{**} + c$ )	$y = 10831 x - 34273$	$y = 97179 x - 25589$
Slope (m)	10831	97179
Intercept (c)	-34273x	-24589x
Correlation coefficient ( $R^2$ )	0.9990	0.9991
LOD ( $\mu\text{g/ml}$ )	0.491	0.522
LOQ ( $\mu\text{g/ml}$ )	1.48	1.58



**Fig. 2: Calibration curve of Imipenem.**



**Fig. 3: Calibration curve of Cilastatin.**

### Precision

System precision and method precision were established by injecting working standard solutions and injection sample solutions at a concentration of 40 µg/ml IPM and CSN, respectively in five replicates. The peak areas were recorded. The relative standard deviation of peak areas of the selected drugs was then calculated to represent precision. The results are summarized in Table 3. The low %RSD values indicated the good precision of the system and method.

**Table No 3: Results of system and method precision.**

System precision			Method precision		
Imipenem					
Amount of drug (µg/ml)	Peak area	Statistical Analysis	Amount of drug (µg/ml)	Peak area	Statistical Analysis
40	403721	<b>Mean:</b> 410765 <b>SD:</b> 61391.54 <b>%RSD:</b> 1.494	40	419676	<b>Mean:</b> 423115 <b>SD:</b> 27435.85 <b>%RSD:</b> 0.648
40	406339		40	423753	
40	411551		40	421920	
40	412655		40	427840	
40	419561		40	423584	
Cilastatin					
40	216473	<b>Mean:</b> 220214 <b>SD:</b> 40693.03 <b>%RSD:</b> 1.847	40	224570	<b>Mean:</b> 226684 <b>SD:</b> 20436.91 <b>%RSD:</b> 0.901
40	216184		40	229140	
40	219842		40	227863	
40	223123		40	223928	
40	225449		40	226740	



### Accuracy

The accuracy of the proposed method was evaluated by recovery experiments via standard addition method. For this purpose, a known amount of standard drugs at three different levels (50%, 100% and 150%) was spiked to the preanalyzed sample. The percentage recovery of the added drugs was calculated. The results of the accuracy of the proposed method are presented in Table 4. From the results obtained, added recoveries of IPM and CSN were found to be accurate.

**Table No 4: Accuracy of the method for Imipenem and Cilastatin.**

Spiked level (%)	Amount of drug		% Recovery	Statistical Analysis of % Recovery
	Added ( $\mu\text{g/mL}$ )	Found ( $\mu\text{g/mL}$ )		
<b>Imipenem</b>				
50	20	20.04	100.75	<b>Mean:</b> 99.69 <b>SD:</b> 0.917148 <b>%RSD:</b> 0.920
	20	19.97	99.31	
	20	20.02	99.02	
100	40	39.88	99.70	<b>Mean:</b> 99.83 <b>SD:</b> 0.4093153 <b>%RSD:</b> 0.410
	40	40.12	100.30	
	40	39.80	99.50	
150	60	60.12	100.21	<b>Mean:</b> 99.97 <b>SD:</b> 0.3099163 <b>%RSD:</b> 0.310
	60	59.76	99.61	
	60	60.06	100.10	
<b>Cilastatin</b>				
50	20	20.04	100.22	<b>Mean:</b> 100.06 <b>SD:</b> 0.180108 <b>%RSD:</b> 0.180
	20	19.97	99.85	
	20	20.02	100.11	
100	40	40.01	100.02	<b>Mean:</b> 100.04 <b>SD:</b> 0.0910364 <b>%RSD:</b> 0.091
	40	40.05	100.14	
	40	39.98	99.96	
150	60	60.08	100.14	<b>Mean:</b> 100.02 <b>SD:</b> 0.090018 <b>%RSD:</b> 0.090
	60	59.97	99.96	
	60	59.98	99.98	

### Robustness

In order to show the robustness of the method, %RSD of peak areas of IPM and CSN were measured at deliberately changed HPLC parameters such as flow rate, column temperature, mobile phase ratio and analytical wavelength. The robustness of the method was evaluated at a concentration of 40  $\mu\text{g/ml}$  IPM and CSN. The results showed (Table.5) that slight variations in method parameters had a negligible effect on the analysis of the selected drugs.

**Table No 5: Robustness of the method for Imipenem and Cilastatin.**

Parameter	Value	Imipenem		Cilastatin	
		Peak area	Statistical Analysis	Peak area	Statistical Analysis
Flow rate (ml/min)	0.9	4037216	Mean: 407204	2245703	Mean: 2271916
	1.0	4063397	SD: 39856.870	2291408	SD: 23582.3892
	1.1	4115511	%RSD: 0.978	2278639	%RSD: 1.037
Temperature (°C)	25	4196762	Mean: 421783	2278639	Mean: 2270259
	27	4237539	SD: 20422.841	2264732	SD: 7379.22870
	29	4219201	%RSD: 0.484	2267407	%RSD: 0.325
Mobile phase ratio (v/v)	78:22	4219201	Mean: 423075	2161848	Mean: 2163837
	80:20	4237216	SD: 10029.155	2198427	SD: 33639.6300
	82:18	4235847	%RSD: 0.237	2131236	%RSD: 1.554
Wavelength (nm)	230	4278401	Mean: 423661	2254490	Mean: 2243809
	232	4235847	SD: 41400.408	2245703	SD: 11742.0467
	234	4195611	%RSD: 0.977	2231236	%RSD: 0.523

**Ruggedness**

In order to show the ruggedness of the method, standard solution of IPM and CSN at a concentration of 40 µg/ml was analyzed by two different analysts, two different columns and two different HPLC systems under the same experimental conditions. The percent recovery and percent RSD were calculated for IPM and CSN. The results showed (Table 6) that variations on this method by analysts, columns and HPLC systems had no significant effect on the analysis of the selected drugs.

**Table No 6: Ruggedness of the method for Imipenem and Cilastatin.**

Parameter	Imipenem				Cilastatin			
	Taken (µg/ml)	Found (µg/ml)	% Recovery	% RSD	Taken (µg/ml)	Found (µg/ml)	% Recovery	% RSD
Analyst I	40	39.90	99.77	0.683	40	39.69	99.22	1.007
Analyst II	40	39.80	99.51	0.213	40	40.10	100.26	0.691
Column I	40	39.61	99.03	0.696	40	39.87	99.67	0.871
Column II	40	39.92	99.80	0.477	40	40.06	100.15	0.285
System I	40	39.81	99.53	0.196	40	40.18	100.45	0.202
System II	40	40.56	101.41	1.345	40	39.46	99.64	1.578

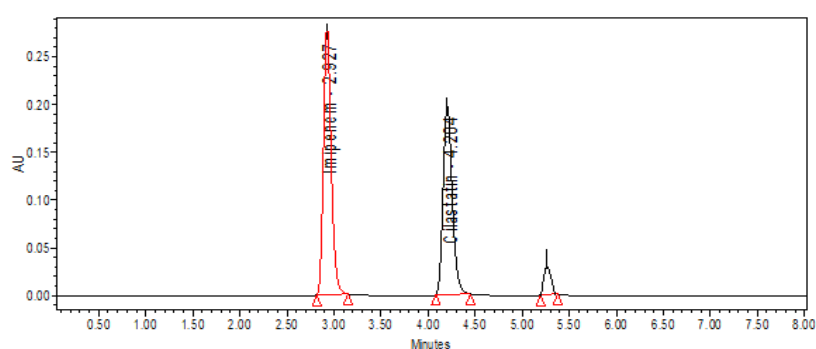
**Stress degradation study**

The specificity of the proposed HPLC method was evaluated by the stress degradation study. The stress degradation study was done to make sure that the proposed method was able to separate IPM and CSN from the degradation products generated during the stress degradation study. The results of the degradation study are presented in Table 7. The chromatograms of degraded samples are shown in Figures 4-8. The degradation products produced due to stress

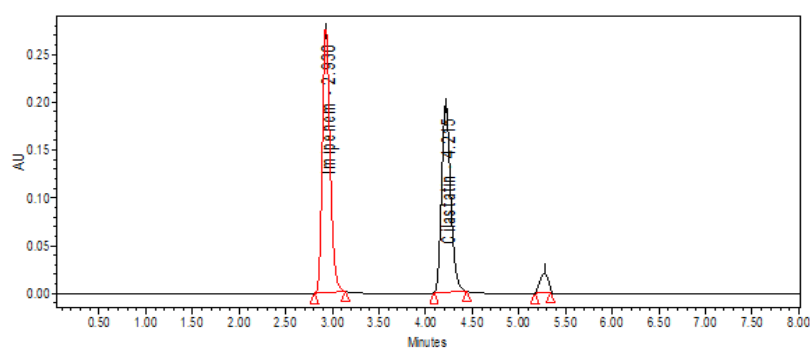
did not interfere with the detection of IPM and CSN. The proposed method can thus be regarded as stability-indicating.

**Table No 7: Results of degradation studies.**

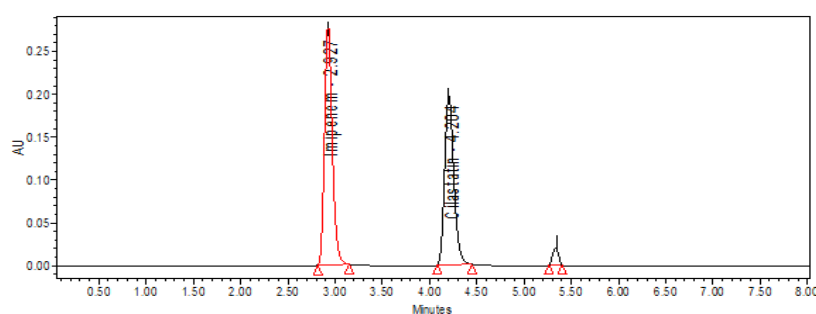
Condition	Imipenem (40 µg/ml)		Cilastatin (40 µg/ml)	
	% Recovery	% Degraded	% Recovery	% Degraded
Acid degradation	95.26	4.74	94.53	5.47
Alkali degradation	96.41	3.59	97.58	2.42
Oxidative degradation	98.24	1.76	96.52	3.48
Photo degradation	98.49	1.51	98.46	1.54
Thermal degradation	98.25	1.75	98.75	1.25



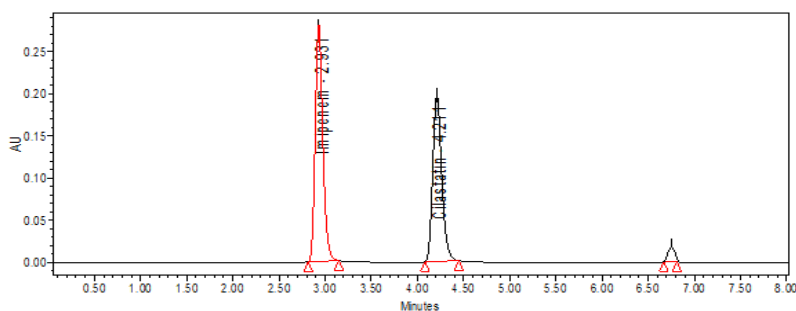
**Fig. 4. Chromatogram of acid degraded Imipenem and Cilastatin.**



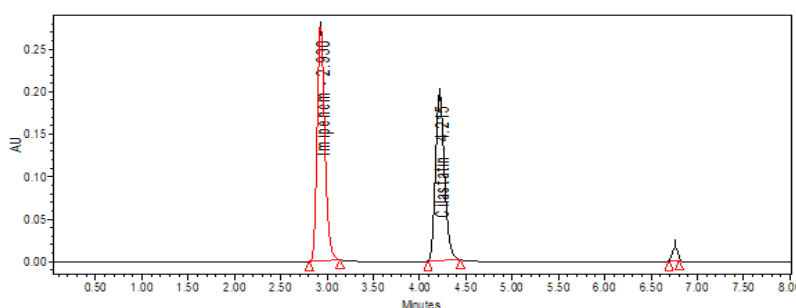
**Fig. 5. Chromatogram of alkali degraded Imipenem and Cilastatin.**



**Fig. 6. Chromatogram of peroxide degraded Imipenem and Cilastatin.**



**Fig. 7. Chromatogram of thermal degraded Imipenem and Cilastatin.**



**Fig. 8: Chromatogram of photo degraded Imipenem and Cilastatin.**

#### APPLICATION OF THE METHOD

The proposed method was applied to the injection formulation containing IPM and CSN at a concentration of 500 mg each per vial. The results shown in Table 8 indicate the high accuracy of the proposed HPLC method for the determination of the studied drugs. The proposed method has the advantage of being virtually free from interferences by excipients. The results were compared statistically with the labeled claim using student t test and F test for accuracy and precision, respectively. The calculated t value and F value are within the tabulated values indicating that the method is accurate and precise for the quantification of IPM and CSN simultaneously in the combined injection dosage form.

**Table No 8: Assay of Imipenem and Cilastatin in injection dosage form.**

Analyte	Labeled claim (mg/ml)	Found (mg)	Mean	% Recovery	% RSD	t-Test <sup>\$</sup>	F-Test <sup>\$\$</sup>
Imipenem	500	500.02	499.99	99.99	0.0736	0.2059	2.0192
	500	500.04					
	500	499.95					
Cilastatin	500	500.08	499.97	99.99	0.0179	0.4085	2.2239
	500	499.98					
	500	499.86					

## CONCLUSION

The developed stability indicating RP-HPLC method has been successfully applied for simultaneous determination of IPM and CSN in their combined injection dosage forms. The method was found to be rapid, simple and accurate. When the developed method was completely validated, the results showed satisfactory data for all the method validation parameters. The low values %RSD, LOD and LOQ indicate the adequate precision and sensitivity of the proposed method. The degradants formed from the stress degradation study did not interfere with the peaks of IPM and CSN indicating the stability indicating the nature of the method. So the proposed method can be easily and conveniently adopted for routine quality control analysis of IPM and CSN.

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