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Research Article

Stability-indicating HPLC method development and validation for simultaneous determination of cilastatin and imipenem in pharmaceutical dosage forms

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ABSTRACT

A combined dosage form of cilastatin and imipenem has been simultaneously determined by adopting an HPLC method. Stability indicating studies have been performed under various stress conditions. The reported method adopts Inertsil ODS C18 (250 mm x 4.6 mm, 5µm) column as stationary phase and a mobile phase consisting of methanol: acetonitrile: acetate buffer in the ratio of 70:25:05(v/v) at a pH 5.2 and an UV detector at 217 nm. Linear calibration curves for proposed method are arrived in the concentration ranges of 1-6 µg/mL for both cilastatin and imipenem (R2 > 0.999 for both drugs). The method is validated in terms of precision, ruggedness, robustness and accuracy. The developed method has been applied successfully to analyse marketed pharmaceutical formulation and vial samples. Degradation studies have been carried out with cilastatin and imipenem by exposing the drugs to various stress conditions like photolytic, aqueous acid, base, thermal and peroxide conditions. The proposed method successfully separated the drug from its degradation products. Hence, it can be employed as stability-indicating method for the determination of cilastatin and imipenem drugs in pure and formulations.

Keywords: HPLC; Cilastatin; Imipenem; Stability-indicating method.

INTRODUCTION

The first member of the carbapenem class of antibiotics called imipenem (Primaxin) is called an intravenous β-lactam antibiotic drug. It shows resistance for β-lactamase enzymes which were produced by several multiple drug-resistant Gram-negative bacteria (Clissold, 1987). It plays a vital role in the treatment of infections which cannot be easily treated with other antibiotics (Vardakas, 2012). Imipenem shows a broad spectrum of activity against aerobic and anaerobic, Gram-positive and Gram-negative bacteria (Kesado, 1980).Burton Christensen, William Leanza, and Kenneth Wildonger discovered the drug in mid-1970 and is found to be active against Pseudomonas aeruginosa and the Enterococcus species. The mechanism of the action of imipenem involves the inhibition of cell wall synthesis of various Gram-positive and Gram-negative bacteria. Cilastatin inhibits the human enzyme dehydropeptidase (Keynan, 1995) which is found in the kidney.It is responsible for degrading the imipenem. Cilastatin helps imipenem to work more effectively by preventing the breakdown of the antibiotic in the kidneys. Cilastatin is therefore combined intravenously with imipenem in order to protect it from dehydro peptidase and prolong its antibacterial effect. Individually, cilastatin does not show any antibacterial activity.

Cilastatin and imipenem combination is found to be an effective antibiotic for the treatment of a number of bacterial infections. This combined drug is used for pneumonia, sepsis, endocarditis, joint infections, intra-abdominal infections and urinary tract infections (Oxford, 2009). The combination is on the World Health Organization's list of essential medicines for the most effective and safe medicines needed in a health system (WHO, 2015). The combination is usually given to the patients by injection into a vein or muscle (Imipenem, 2016; Hamilton, 2015). The review of literature indicates that very few liquid chromatographic methods are available to analyse the cilastatin and imipenem. Among them the methods have been reported for selected drugs for combination assay (Sandhya Rani,2017;Syed Imram, 2016; Srinivasan, 2014; Satheesh thotla, 2015), combination bio-analytical (Carolyn, 1984; Rea Krausse, 1986), combination UV-derivative (Forsyth, 1994; Omar Samir Tabbouche, 2014), combination with impurities UPLC (PrathyushaVikram, 2016), imipenem bio-analytical (Walter, 1988), imipenem single visible (Raghu Babu, 2014a ; 2014b), single spectrophotometric and RP-HPLC (Raghu Babu, 2014c). However, no stability

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indicating RP-HPLC method has been reported so far and hence forms the basis of the present work.

MATERIAL AND METHODS

Instrumentation

PEAK chromatographic system equipped with LC-P7000 isocratic pump and a UV detector UV7000 was adopted in the analysis, a software version of 1.06 of the company were used for following the output signal. Rheodyne injector was used with 20 µL fixed volume loop for sample injection .Denver electronic analytical balance (SI-234) was used for weighing the samples. Systronics digital pH meter was used for adjusting the pH of the mobile phase.

Chemicals and Solvents

The pharmaceutical formulation was procured from local market. HPLC grade methanol, acetonitrile, buffer solutions obtained from Merck Specialties Private Limited, Mumbai, India was used.

Preparation of Standard and Sample Solution

Stock solutions of the two standard drugs were prepared individually by dissolving 10 mg of each in to 10 mL of solvent in a volumetric flask to prepare a concentration of 1000 µg/mL. Different concentrations of solutions were prepared by proper dilution. Sample solution was prepared by mixing 10 formulation vials of imipenem and cilastatin (IMICRIT; cilastatin-500 mg and imipenem–500 mg) and the solution equivalent to 10 mg of cilastatin was weighed accurately and diluted properly. Then the solution was filtered and made up to 10 mL with same diluents to make 1000µg/mL stock solution. From this, by proper dilution a concentration of 3 µg/mL of cilastatin was prepared. As per the label claim of the two drugs, imipenem concentration of 3 µg/mL was obtained. The resultant solution was used for the subsequent simultaneous determination of cilastatin and imipenem in combined dosage forms.

Various chromatographic conditions have been optimized in order to achieve separation and identification of imipenem and cilastatin. As per USP and ICH guidelines, the developed method was validated in terms of system suitability, specificity, linearity, precision, accuracy, LOD, LOQ, ruggedness, robustness and solution stability.

Forced Degradation Studies

Stress degradation studies were made by subjecting 50 mg of the drug to acidic, alkaline, oxidizing, thermal and photolytic conditions. The drug was heated under reflux with 0.1 M HCl and 0.1 M NaOH at 80ºC for 2 h and the mixture was neutralized respectfully for studying acidic and alkali degradation. For oxidizing degradation, the drug was heated under reflux with (30%, v/v) H_2O_2 at 80ºC for 2 h. The powdered drug was exposed at 70ºC for 48 h for thermal degradation. Photolytic degradation was carried out by exposing the powdered drug to the sunlight for two days. The placebo was also treated in the similar way to monitor any peaks to be observed for the excipients in the dosage forms under stress conditions. After completion of the treatments by allowing the solutions to return to room temperature, sufficient quantity of solvent mixture was added to obtain 30 µg/mL. The quantity of the drug peak obtained from the stressed sample was measured and compared with the chromatogram of untreated drugs in tablet solution.

Method Development

In order to develop a stability indicating RP-HPLC method for the analysis of the cilastatin and imipenem in combined form, a study was carried out under isocratic condition by performing several tests such as changing the mobile phase composition with various ratios of organic phase and buffers, different lengths of C18 columns to get satisfactory resolution of cilastatin and imipenem. A mobile phase containing methanol: acetonitrile: acetate buffer in the ratio of 70:25:05 (v/v) at pH 5.2 was proved to be the most suitable of all combinations since the peaks meet all the specifications of system suitability conditions. UV detector with 217 nm was selected for detection. Pump flow was set at 1.3 mL/min. Inertsil ODS C18 (250 mm x 4.6 mm, 5 µm) column was found to be suitable for separation. The flow rate was isocratic and retention time of cilastatin and imipenem were observed to be 5.83 min, 10.57 min respectively.

Method Validation

ICH guidelines (Q2A) were followed in developing the stability indicating HPLC method for the determination of cilastatin and imipenemin combined form. The validation parameters include system suitability, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), sensitivity, selectivity and specificity. The method was found to show linearity in the concentration range of 1-6 µg/mL for both cilastatin and imipenem (n=6) by following the regression equation y = 10761x + 11055, y = 95313x + 15726 with correlation coefficient of 0.999for the regression lines indicating that the method was linear over a considerable range and could be useful for pharmaceutical analysis of the selected combination of drugs.

The specificity of the method was ascertained by comparing the chromatograms of the tablet, blank solution (mobile phase) and standard drug solution. The generated chromatograms were compared with the chromatogram of individual drugs. No extraneous peaks were found with the same retention time of the drugs. Thus, the method was found to be specific for the analysis of cilastatin and imipenem. The standard chromatogram was given in Figure 2. The accuracy was established by applying the proposed method to the synthetic mixtures of formulation excipients to which known amount of cilastatin and imipenem were added.

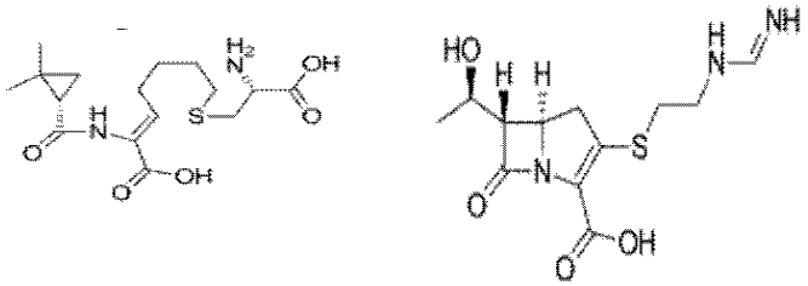

Figure 1: Chemical structure of cilastatin and imipenem

Table 1: Optimized chromatographic conditions

S.NO	Parameter	Results
1	Mobile Phase	Methanol: Acetonitrile: Acetate buffer70:25:05(v/v)
2	Wavelength	217 nm
3	Stationary Phase	Inertsil ODS C18 (250 mm x 4.6 mm, 5µm) column
4	pH of Mobile Phase	5.2
5	Flow Rate	1.30 mL/min
6	Pump Mode	Isocratic
7	Pump Pressure	11.5±5MPa

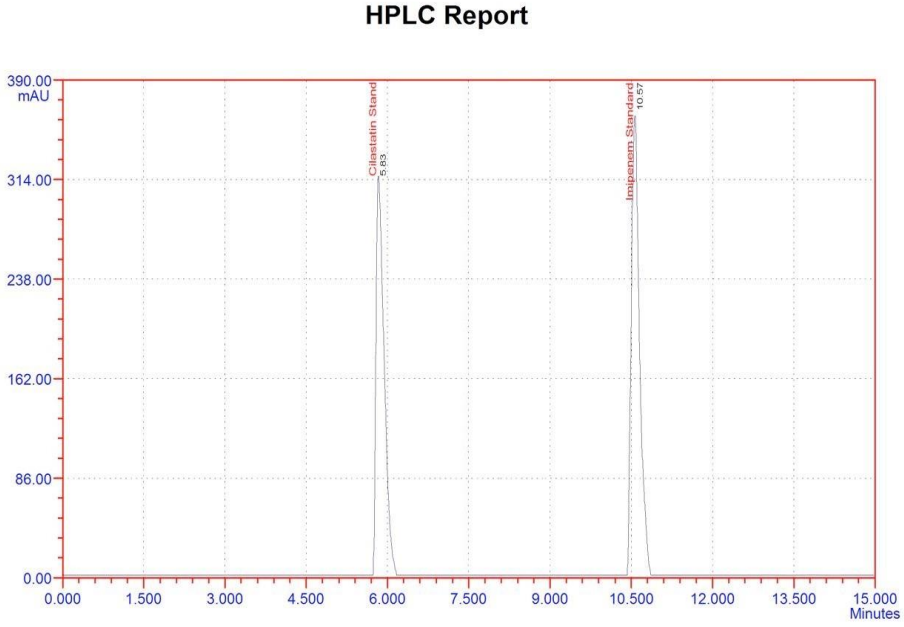

Figure 2: Standard chromatogram of cilastatin and imipenem in the optimized condition

Table 2: System suitability conditions

S.NO	Parameter	Results
1	Active Pharma ingredient Concentration	Cilastatin – 3 µg/mL Imipenem–3 µg/mL
2	Retention Time	Cilastatin – 5.83 min Imipenem -10.57 min
3	Resolution	Cilastatin– ……. Imipenem – 18.9
4	Area	Cilastatin –307197 Imipenem - 322967
5	Theoretical Plates	Cilastatin – 6418 Imipenem - 24130
6	Tailing Factor	Cilastatin – 1.87 Imipenem - 1.47

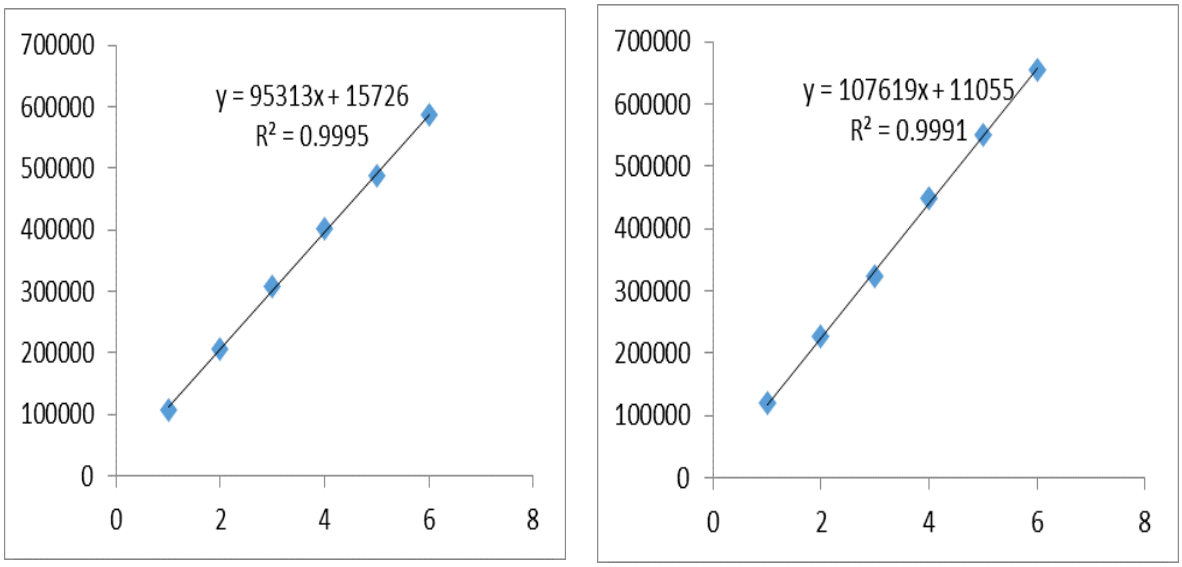

Figure 3: Linearity graph for imipenem and cilastatin

Table 3: Validation results

Parameter	Results	
	Cilastatin	Imipenem
Linearity range (µg/mL)	1-6 µg/mL	1-6 µg/mL
Correlation coefficient	0.999	0.998
Slope	95313	10761
Intercept	15726	11055
LOD (µg/mL)	0.003 µg/mL	0.003 µg/mL
LOQ (µg/mL)	0.01 µg/mL	0.01 µg/mL
Recovery (%)	98.859-100.205	98.187-101.715
Precision (RSD %)		
Intraday (n=6)	0.419	0.888
Interday (n=6)	0.541	0.824
Ruggedness (n=6)	0.557	0.764
Robustness (% Change)		
Mobile phase 1 (Methanol: Acetonitrile: Acetate buffer 72:23:05 (v/v))	0.410	1.965
Mobile phase 2 (Methanol: Acetonitrile: Acetate buffer 68:27:05 (v/v))	0.769	0.805
pH 1 (5.4)	0.969	0.959
pH 2 (5.0)	1.556	0.822
Wave Length 1 (219 nm)	0.880	0.221
Wave Length 2 (215 nm)	1.385	0.7242
Formulation assay	98.8939	99.7907

Table 4: Summary of degradation results

Condition	No of additional peaks observed	Cilastatin			Imipenem		
		Area Obtained	% Recovered	% Degradation	Area Obtained	% Recovered	% Degradation
Acid	3	299251	97.41	2.59	301204	93.26	6.74
Base	3	285216	92.84	7.16	302985	93.81	6.19
Light	4	298395	97.13	2.86	312878	96.88	3.12
Peroxide	4	297158	96.73	3.27	308150	95.41	4.59
Thermal	2	293935	95.68	4.32	313728	97.14	2.86
UV	3	295889	96.32	3.68	310504	96.14	3.86

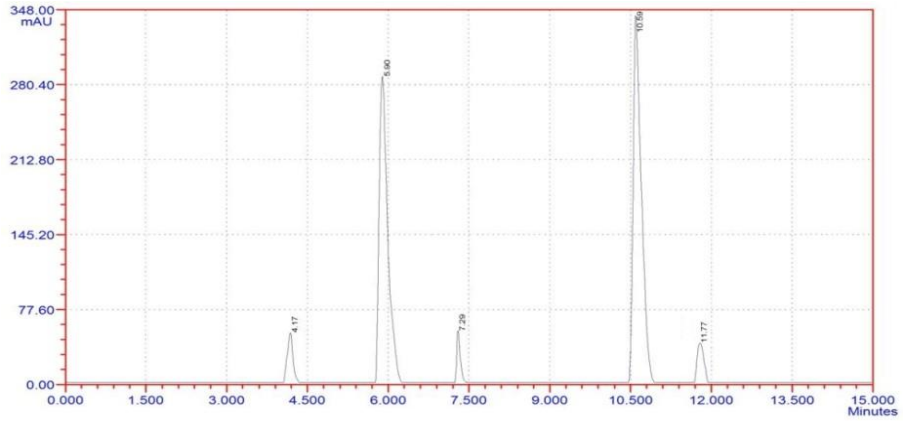

Figure 4: Acid degradation chromatogram

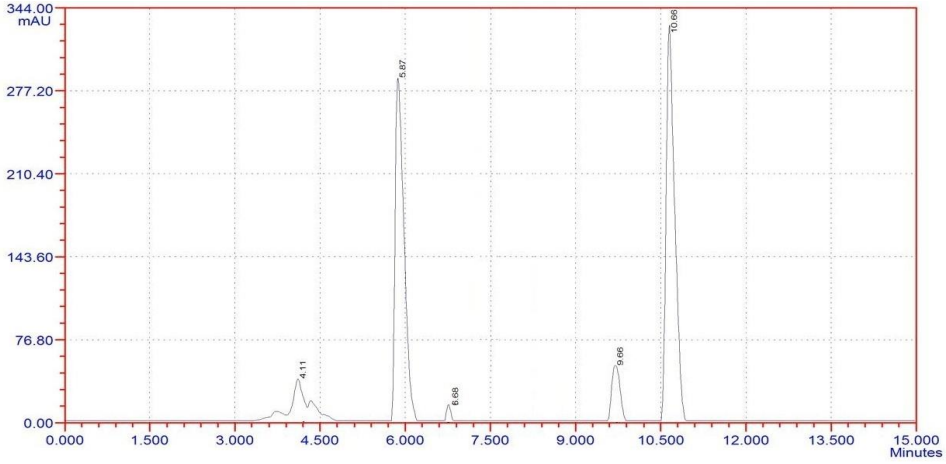

Figure 5: Base degradation chromatogram

HPLC Report

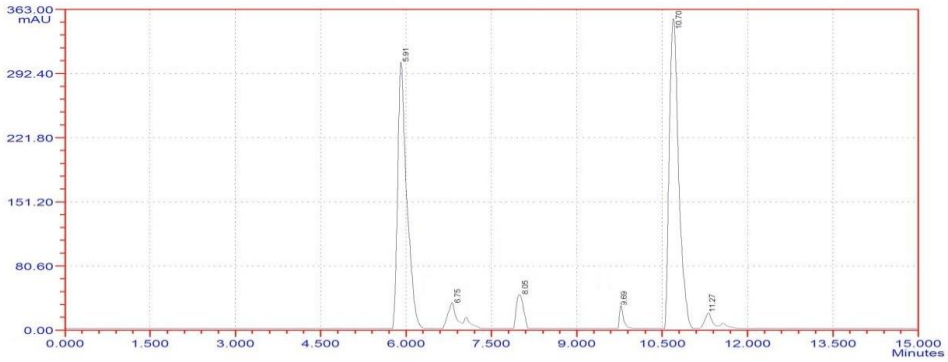

Figure 6: Peroxide degradation chromatogram

HPLC Report

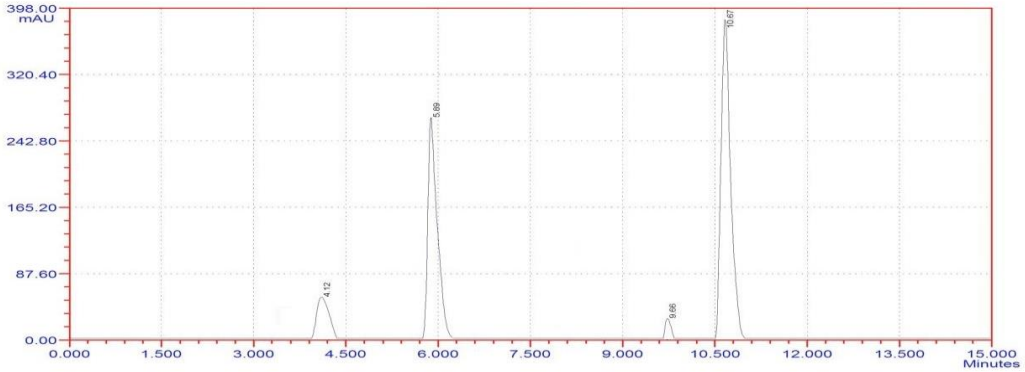

Figure 7: Thermal degradation chromatogram

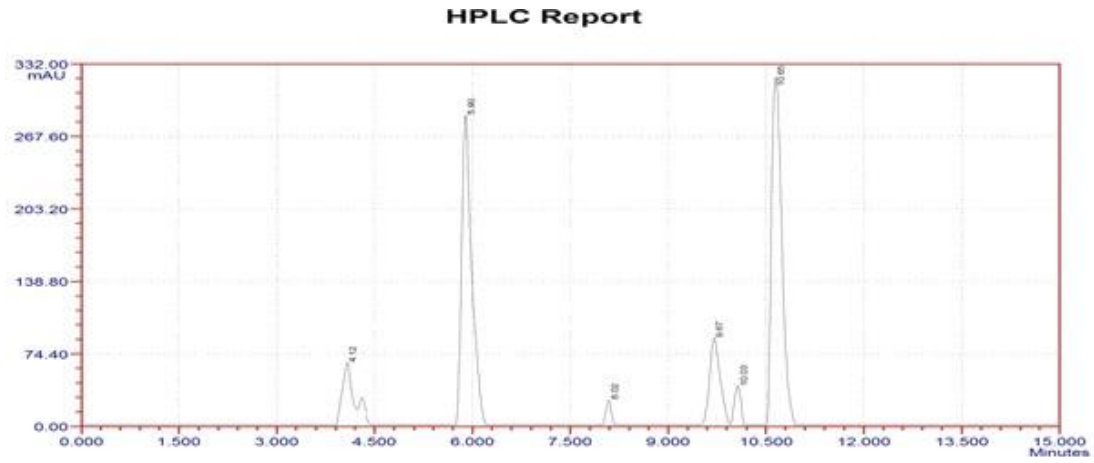

Figure 8: Light degradation chromatogram

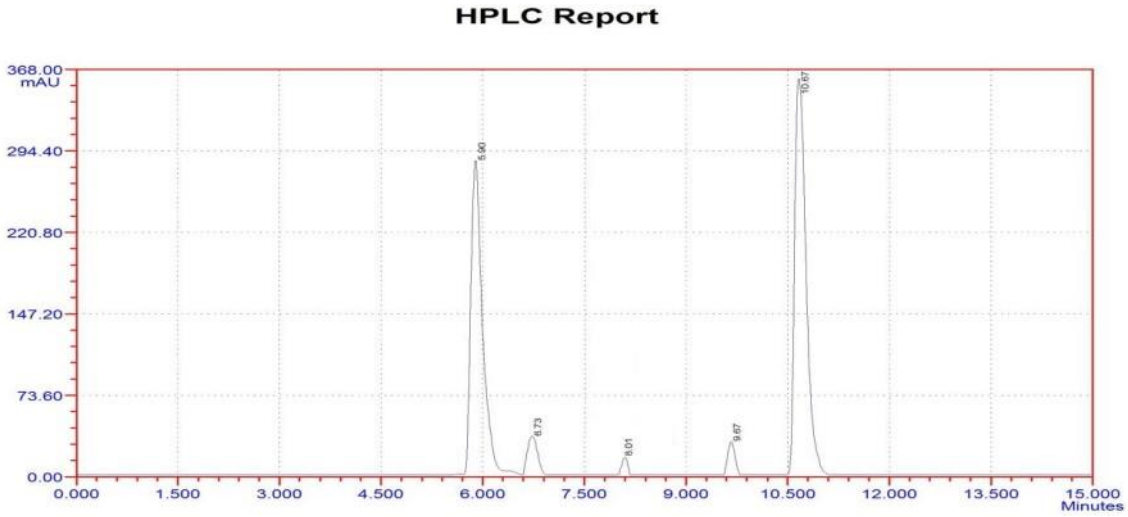

Figure 9: UV Light degradation chromatogram

Mean recovery for cilastatin was found to be in between 98.86-100.20% and 98.19-101.71% for imipenem indicating that the accuracy of the developed method is good. Other important parameters like precision, ruggedness and robustness results were also found to be within the limits. The percentage RSD for cilastatin at 3 µg/mL were found to be 0.42, 0.54 and 0.56 for intraday, interday and ruggedness studies; and percentage RSD of imipenem was found to be 0.89, 0.82 and 0.76 for intraday, interday and ruggedness respectively. The percentage of change in results for robustness study includes 0.41-1.55% for cilastatin and 0.22-1.96% for imipenem. LOQ and LOD were observed to be 0.01 µg/mL and 0.003 µg/mL for both the drugs respectively.

The drugs cilastatin and imipenem in commercially available IMICRIT© tablets have been determined with the reported validated HPLC method and the results were found to yield 98.89 and 99.79% of label claim respectively. The results of the assay indicate that the method is selective for the routine analysis of cilastatin and imipenem with no chromatographic interference from the excipients used. Table 3 provides the summary of validated results.

A forced degradation study was performed on the combined form of cilastatin and imipenem drugs at different stress conditions. The chromatograms of samples degraded with acid, base, hydrogen peroxide and light showed well separated peaks of pure cilastatin and imipenem as well as some additional peaks at different t_R values. Among all studied conditions, peroxide and sunlight conditions are found to be effective for both drugs whereas four additional degraded compounds were found separately. Thermal conditions were found to be stable to the selected drugs where only two degraded peaks were identified. Three additional peaks were separated in the remaining forced degradation conditions like acid, base, and ultraviolet condition. Stability studies chromatograms were presented in Figures (4-9). The percentage recovery in the degradation studies was also carried out for cilastatin and imipenem in the optimized method. More than 90% recovery was obtained for both the drugs in all the degradation conditions. Hence, the method was considered to be

stable in all the stress degradation conditions studied. Results were given in Table 4.

CONCLUSION

The reported isocratic HPLC method for the determination of cilastatin and imipenem has been evaluated for system suitability, linearity, precision, accuracy, stability of solutions, and specificity. Quantification was achieved with ultraviolet detection at 217 nm. The retention time obtained for cilastatin was at 5.83 min and for imipenem was at 10.57 min. The detector response was found to be linear in the concentration range of 1-6 µg/mL for both cilastatin and imipenem. The proposed method was found to be accurate, precise, and robust. The degradation products were resolved from the pure drug with significantly different retention time values. The method can be successfully applied for the determination of active pharmaceutical ingredient (API) present in the combined dosage forms of cilastatin and imipenem.

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