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A HIGHLY SENSITIVE STABILITY-INDICATING UPLC METHOD FOR THE DETERMINATION OF RELATED SUBSTANCES IN IMIPENEM AND CILASTATIN FOR INJECTION

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ABSTRACT

A simple, sensitive and specific RP-UPLC method was developed for the quantification of related impurities of Imipenem and Cilastatin in liquid pharmaceutical dosage form. The chromatographic separation employs a gradient elution using a waters Sunfire C18, 75mm×3.0mm, 2.5µm columns. Mobile phase consisting of solvent A (solution containing 0.88 g of potassium dihydrogen phosphate and 0.44 g of dipotassium hydrogen phosphate per liter of water, adjusted to pH 7.0 with sodium hydroxide)and solvent B(acetonitrile) delivered at a flow rate of 0.5 ml min−1. The analytes were detected and quantified at 215nm using photodiode array (PDA) detector. The method was validated as per ICH guidelines, demonstrating to be accurate and precise (repeatability and intermediate precision level) within the corresponding linear range of known impurities of Imipenem and Cilastatin. The specificity of the method was investigated under different stress conditions, including hydrolytic, oxidative, photolytic and thermal as recommended by ICH guidelines. Robustness against small modification in pH, column oven temperature, flow rate and percentage of the mobile phase composition was ascertained. All these results provide the stability indicating capability of the method.

KEY WORDS : ultra performance liquid chromatography (UPLC) ; Imipenem ; cilastain ; degradation products ; stability sample analysis.

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INTRODUCTION

Imipenem/Cilastatin [(5R,6S)-6-[(1R)- 1-hydroxyethyl]-3-({2[(iminomethyl)amino]ethyl}thio)-7-oxo -1-azabicyclo [3.2.0]hept-2-ene -2-carboxylicacid/ (Z)-7- [(2R)-2-amino-3 hydroxy-3oxopropyl] sulfanyl -2- {[(1S)-2,2dimethylcyclopropanecarbonyl]amino}hept-2-enoic acid] is a broad spectrum beta-lactam antibiotic containing equal quantities of Imipenem and Cilastatin.Imipenem[1]is related to the penicillin/cephalosporin family of antibiotics, but is classified as belonging to the carbapenem class. Imipemen is an off-white, nonhygroscopic crystalline compound with a molecular weight of 317.37. It is sparingly soluble in water, and slightly soluble in methanol. Its empirical formula is $C_{12}H_{17}N_3O_4S.H_2O$. Cilastatinis a renal dehydropeptidase-I and leukotriene D4 dipeptidase inhibitor. Since the antibiotic, Imipenem, is hydrolyzed by dehydropeptidase-I, which resides on the brush border of the renal tubule, Cilastatin is administered with Imipenem to increase its effectiveness. Cilastatin is off-white to yellowish-white, hygroscopic, amorphous compound with a molecular weight of 380.43. It is very soluble in water and in methanol. Its empirical formula is $C_{16}H_{25}N_2O_5SNa$. To date, all analytical methods described in literature [Table-1] include determination of Imipenem and Cilastatin are by UV derivatization[2,3]determination of Imipenem in combination of different molecules in biological fluids involve liquid chromatography[4,5]determination of only Imipenem in biological fluids involve liquid chromatography[6,7]and determination of only Cilastatin in biological fluids involve liquid chromatography[8]. However the exhaustive literature survey revealed that none of the most recognized pharmacopoeias or any journals include these drugs in combination for the simultaneous determination of related substances of Imipenem and Cilastatin and the information regarding the stability of the drugs is not available. So, it is felt essential to develop a liquid chromatographic procedure which will serve a reliable, accurate, sensitive and stability indicating UPLC method for the simultaneous determination of related substances of Imipenem and Cilastatin liquid injectables.

Table 1

Comparison of the performance characteristics of the present method with the published methods

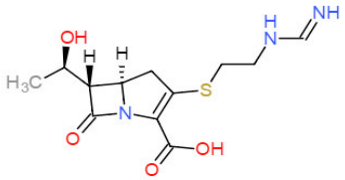
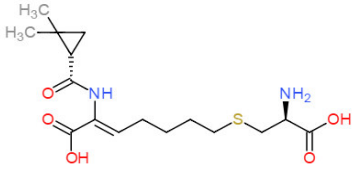
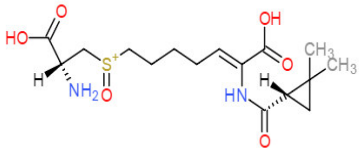
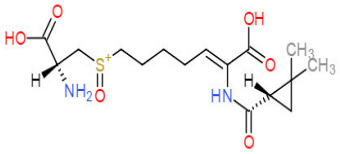
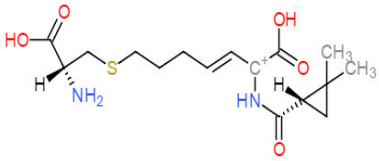
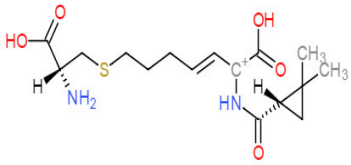
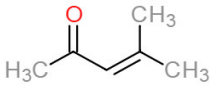
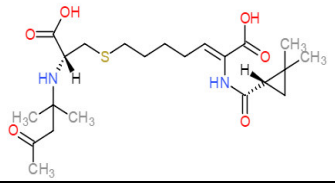
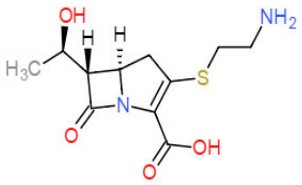
S.NO	Method	λ (nm)	Remark	Reference
1	UV/Imipenem & Cilastatin	243 &318	Dervitization method	2
2	UV/Imipenem & Cilastatin	243 &318	very narrow linearity range	3
3	HPLC/Imipenem&Sulbactum	-	Mouse Plasma	4
4	HPLC/Imipenem & Rifampicin	300 &255	Mouse Plasma	5
5	HPLC/Imipenem	300	Human Plasma	6
6	HPLC/Imipenem	275	Human serum	7
7	HPLC/Cilastatin	-	Biological Fluids	8
8	UPLC/ Imipenem & Cilastatin	215	Stability Indicating wide linearity Range	Present Work

MATERILAS AND METHODS

(i) Reagents and materials

Imipenem and Cilastatin active pharmaceutical ingredient (API) and related substances of Imipenem and Cilastatin(Fig. 1) were procured from Orchid chemicals &Pharmaceuticals, Chennai, India. CILIVA 500 (Imipenem & Cilastatin for injection 500mg) test sample solution was purchased in the commercial available market. Potassium dihydrogen orthophosphate, dipotassium hydrogen phosphate, Orthophosphoricacid, sodium hydroxide pellets and HPLC grade acetonitrile were obtained from Merck Limited, Mumbai, India. High purity deionised water was obtained from Millipore, Milli-Q (Bedford, MA, USA) purification system.

Figure 1
Structure of Imipenem& Cilastatin and its Impurities

S.No	Name	Mass	Structure
1	Imipenem	317.37	
2	Cilastatin	380.43	
3	EP impurity-A_1	374	
4	EP impurity-A_2	374	
5	In-House impurity-1	358	
6	In-Houseimpurity-2	358	
7	EP Impurity-D	98	
8	EP Impurity-C	456	
9	Thienamycin	272	

(ii) Instrumentation

UPLC H-class system (Waters Milford, USA) equipped with inbuilt auto sampler and quaternary gradient pump with an on-line degasser was used. The column compartment having temperature control and photodiode array (PDA) detector was employed throughout the analysis. Chromatographic data was acquired using empower2 software.

(iii) Chromatographic conditions

Waters Sunfire C18, 75mm×3.0mm, 2.5µm(GL sciences Inc., USA) column was used as stationary phase maintained at 40°C. The mobile phase involved a variable composition of solvent A solution containing 0.88g of potassium dihydrogen phosphate and 0.44g of dipotassium hydrogen phosphate per liter of water, adjusted to pH 7.0 with 0.5N sodium hydroxide) and solvent B(acetonitrile).The mobilephase was pumped through the column with at a flow rate of0.5mlmin−1 (Table 2).The optimum wavelength selected was 215nm which represents the wavelength of maximum response for all impurities in order to permit simultaneous determination of related impurities of Imipenem and Cilastatin. The stressed samples were analyzed using a PDA detector covering the range of 200–400nm.

Table 2
Mobile phase program for gradient elution

Time (min)	Flow (mlmin−1)	Solvent A (%)	Solvent B (%)
0	0.5	100	0
2	0.5	100	0
3	0.5	98	2
4	0.5	95	5
6	0.5	92	8
13	0.5	92	8
18	0.5	90	10
20	0.5	85	15
23	0.5	78	22
25	0.5	73	27
26	0.5	73	27
28	0.5	100	0
30	0.5	100	0

(iv) Solution preparation

a. Preparation of buffer and diluent

About 0.88g of potassium dihydrogen phosphate and 0.44g of dipotassium hydrogen phosphate dissolved in 1000mL of water, adjusted to pH 7.0 ±0.05 with 0.5N sodium hydroxide solution was used as buffer. About 0.135 g of potassium dihydrogen phosphate dissolved in 900 mL of water, adjusted to pH 6.8 ± 0.05 with 0.5N sodium hydroxide solution and diluted to 1000mL with water was used as diluent.

b. Standard solution

A stock solution of Imipenem and Cilastatin (500 µg/ mL each) was prepared by dissolving an appropriate amount in the diluent. Solution containing a mixture of 5µg/mL of Imipenem and Cilastatin each was prepared from stock solution, used as working standard.

c. Sample solution

Reconstituted Imipenem Cilastatin 500mg with 10mLof saline solution, a stock solution of Imipenem Cilastatin(5mg/mL) was prepared by using saline solution. The stock solution diluted accordingly to give a solution containing 500µg/ mL as sample solution.

d. Forced degradation sample solution for specificity study

The study was intended to ensure the separation of Imipenem Cilastatin and its degradation impurities. Forced degradation study was performed to evaluate the stability indicating properties and specificity of the method[9]. Multiple stressed samples were prepared as indicated below. They were chromatographed along with a non-stressed sample. *Hydrolytic conditions: acid-, base-, water- induced degradation. Acid*-Solution containing 0.5mg/mL of Imipenem and Cilastatin was treated with 0.1N HCl for 5min on bench top. The solutions were neutralized with 0.1N NaOH accordingly. *Base*- Solution containing 0.5mg/mL of Imipenem and Cilastatin was treated with 0.1N NaOH for 2min on bench top. The solutions were neutralized with 0.1N HCl accordingly. *Water*- Solution containing 0.5mg/mL of Imipenem and Cilastatin was treated with water at 70°C for 10min. *Oxidative condition: hydrogen peroxide-induced degradation.* Solution containing 0.5mg/mL of Imipenem and Cilastatin was treated with 1% w/v H_2O_2on bench top for 2min.

Humidity degradation study.

Solution containing 5mg/mL of Imipenem and Cilastatin was exposed to 25°C/ 90% RH for 4hours.5 ml of the above solution was transferred in 50 ml volumetric flask, diluted to the volume with diluents

Photolytic degradation study.

The drug solution of Imipenem Cilastatin (5mg/mL) was exposed to the UV light in the photolytic chamber for 24hrs. 5ml of the above solution was transferred in 50ml volumetric flask, diluted to the volume with diluent.

RESULTS AND DISCUSSION

(i) Optimization of chromatographic conditions

a. Selection of mobile phase:

Considering that Imipenem Cilastatin and their related compounds basic buffer in combination of organic modifiers was selected, following mobile phases with gradient elution were tested,

1. $KH_2PO_4 \cdot H_2O$ (10mM) andK_2HPO_4(5mM) as a buffer (pH 6, 6.5, 7, 7.5,) in combination with acetonitrile.
2. $(NH_4)H_2PO_4$(10mM) and $(NH4)_2HPO_4$ (5mM) as a buffer (pH 6.0, 6.5, 7.0, 7.5,) in combination with acetonitrile.
3. $KH_2PO_4 \cdot H_2O$ (5mM) and K_2HPO_4 (2.5mM) as a buffer (pH 6.5, 7.0) in combination with acetonitrile
4. $KH_2PO_4 \cdot H_2O$ (5mM) and K_2HPO_4 (2.5mM) as a buffer (pH 6.5, 7.0) in combination with methanol

5. $KH_2PO_4 \cdot H_2O$ (6.5mM) and K_2HPO_4 (2.5mM) as a buffer (pH 6.5, 7, 7.5,) in combination with acetonitrile
6. $KH_2PO_4 \cdot H_2O$ (6.5mM) and K_2HPO_4 (2.5mM) as a buffer (pH 6.5, 7.0, 7.5,) in combination with methanol.

Trails with different combination of buffer and organic modifier were performed,Ammonium phosphate buffers have given broad peak shape for Cilastatin and EP Impurity A1 & A2 are completely merging with each other.Potassium phosphate buffers found to be having good peak shape but with 10mM$KH_2PO_4 \cdot H_2O$ strength of the buffer In House Impurity 1 is merging with Cilastatinand with the lower strength (5mM $KH_2PO_4 \cdot H_2O$)of buffer broader peak shapes of EP Impurity C was observed. Hence $KH_2PO_4 \cdot H_2O$ (6.5mM) and K_2HPO_4 (2.5mM) as a buffer (pH 7.0) in combination with acetonitrile was chosen to improve resolution among the impurities and peak shapes of Cilastatin.

b. Selection of stationary phase

The possible impurities of Imipenem and Cilastatin are very similar to respective drug substances. To obtain a good resolution among the impurities and main drug substances different stationary phases were tested considering;

- The feature of stationary phase (C8 and C18).
- The particle size of the column (1.7µm and 3µm).

It is clear from the molecular structure (Figure. 1) that all compounds do not possess a functional group which can readily ionize indicating non-polar in nature. Hence we started the development activity with C8 stationary phase of various manufacturers using different mobile phases. The poor resolution between Imipenem and Theinamycin, EP Impurity-A1&A2 and broad peak shape for Cilastatin implies that C8 stationary phase is not suitable for this application. Hence C18 stationary phase was chosen to improve resolution among the peaks and peak shape for Cilastatin. The development activity was further proceeded with Waters acquity C18 100mmx 2.1mm 1.7µ column, the Imipenem and The inamycin were completely merged with each other, very poor resolution was observed between In House Impurity 1& 2 implies that 2.1µm column is not suitable for this application. Hence 2.5µm column was used to improve resolution among all components, with Waters Sunfire C18, 75mm×3.0mm, 2.5µm column the peak shapes and resolution among all components was found to be satisfactory. The stationary phase is not only the parameter which can give better separation among all impurities. Mobile phase, pH and organic modifies also plays very important role which leads to the best separation. Hence in order to get the best separation among the impurities and main peaks the further trails were proceeded with Waters Sun fire C18, 75mm×3.0mm, 2.5µm column as stationary phase.

c. Influence of organic modifier

Initially the methanol was used as an organic modifier which gives the poor baseline with baseline drift. Hence the response for the related compounds was reduced. The retention for all impurities was increased leading to poor resolution among the peaks. To improve the resolution among the peaks and response, acetonitrile was tried as an organic modifier. The base line was found good and response for all components was improved. The peak shape for all components was also improved and hence acetonitrile was selected as organic modifier. The composition of the acetonitrile was altered [Table 2] accordingly depending on the molecule and impurity to obtain the best separations among the impurities and main peaks.

d. Influence of pH of mobile phase buffer

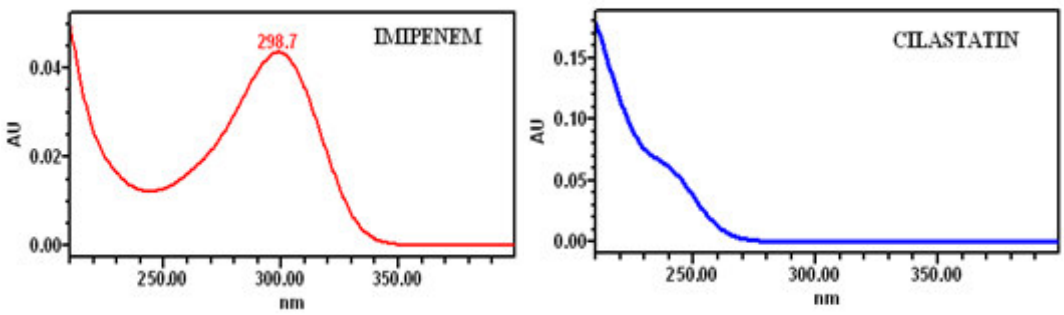
Different pH values of the mobile phase were checked to establish the optimum separation and the highest analytical sensitivity for Imipenem & Cilastatin and its degradation products. The mobile phase was buffered because of the existence of ionizable groups in the chemical structure of the drug, which could ionize at different pH values. The pH values tested were 6.5, 7.0 and 7.5. Finally, the best results were obtained at pH 7.0 ±0.1 by adjusting with 0.5N sodium hydroxide solution. The choice of this mobile phase is justified by the excellent symmetry of the peaks and adequate retention times of Imipenem &Cilastatin and its degradants.

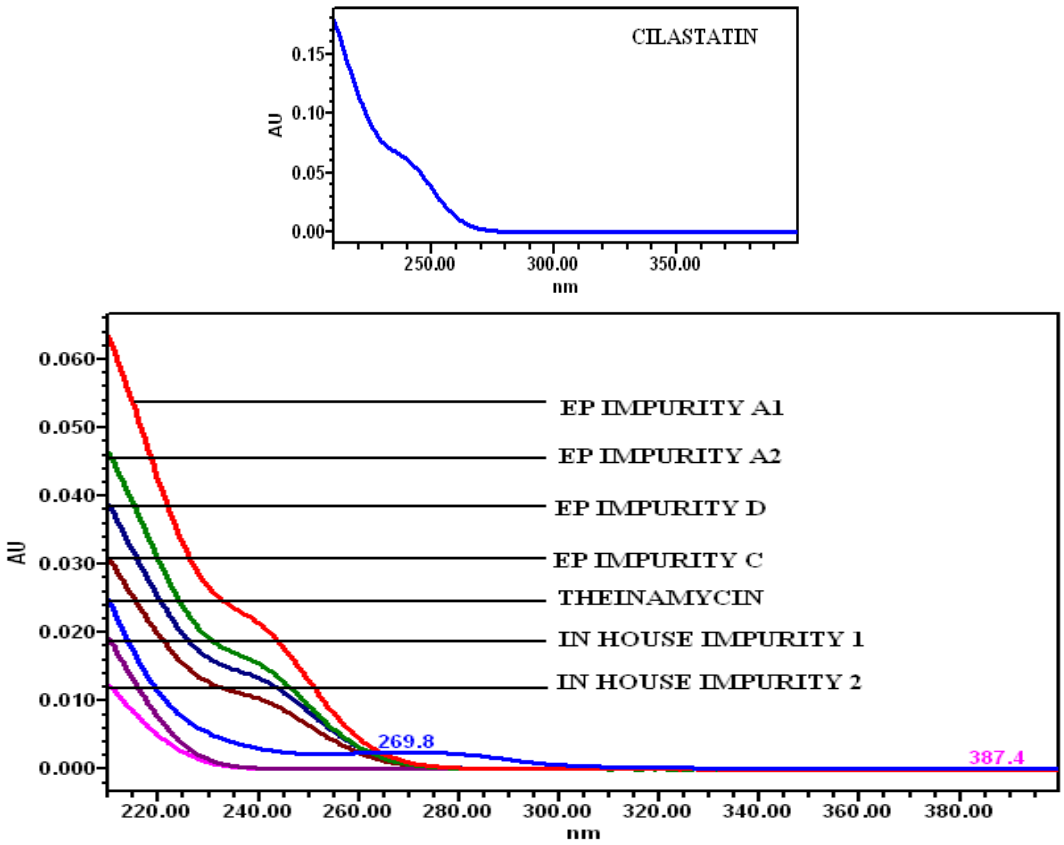
e. Selection of wavelength

Based on the spectra of Imipenem - Cilastatin and its related substances 215 nm was selected as detection wavelength for the method. (Figure-2).

Figure 2

Spectra of Imipenem Cilastatin and its related impurities





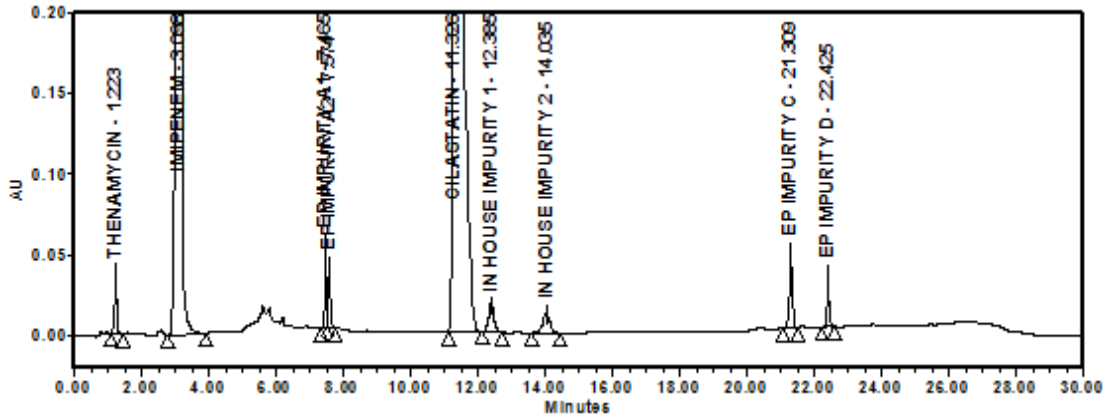
f. Flow rate optimization

Different mobile phase flow rates (0.4, 0.5and 0.6 mL min-1) were investigated. The optimum flow rate for which the column plate number was maximum, with the best resolution between all compounds and with a runtime of 30 min observed was 0.5mLmin-1.

g. Column temperature optimization

Column thermostat temperatures were used at 35ºC, 40ºC and 45ºC for better peak shapes, baseline and resolution. At the column oven temperature of 40ºC the finest baseline resolution was observed between all the components After an extensive study, the method has been finalized on waters Sun fire C18, 75mm×3.0mm, 2.5µm columns using variable composition of solvent A: KH_2PO_4 (6.5mM), K_2HPO_4(2.5mM) pH 7.0 with sodium hydroxide and solvent B: acetonitrileas mobile phase. The mobile phase pumped through the column with gradient elution (Table 2)at a flow rate of 0.5ml min−1 and column compartment temperature kept at 40°C. Depending on the λ max (Figure. 2) of the Imipenem & Cilastatin and its related impurities the detector response for all the components found maximum at 215 nm, hence the typical chromatogram was recorded at this wavelength. The typical UPLC chromatograms (Figure. 3) represent the satisfactory separation of all components among each other.

Figure 3
Spiked chromatogram



(ii) Results of specificity/ Force degradation study

The peak purity indices for the analytes in stressed solutions were determined with PDA detector under optimized chromatographic conditions found to be better (purity angle < purity threshold) indicating that no additional peaks were co-eluting with the analytes and evidencing the ability of the method to assess unequivocally the analyte of interest in the presence of potential interference. Baseline resolution was achieved

for all investigated compounds. The FDA guidelines indicated that well separated peaks, with resolution, Rs > 2 between the peak of interest and the closest eluting peak, are reliable for the quantification[10].Degradation was not observed in Imipenem - Cilastatin sample during photolytic, water, oxidative and humidity stress. About 1.02% and 2.09% of degradation was observed in acid and base stress respectively. The method is linear in the tested range. Peaks meet this specification, visibly confirmed in Figure 4.

Figure 4
Force degradation/Specificity study
Figure 4.1
Acid stress

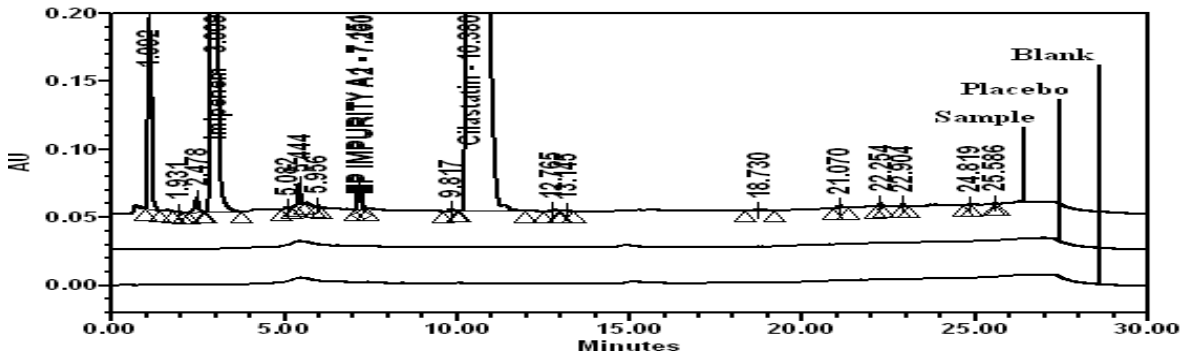


Figure 4.2
Base stress

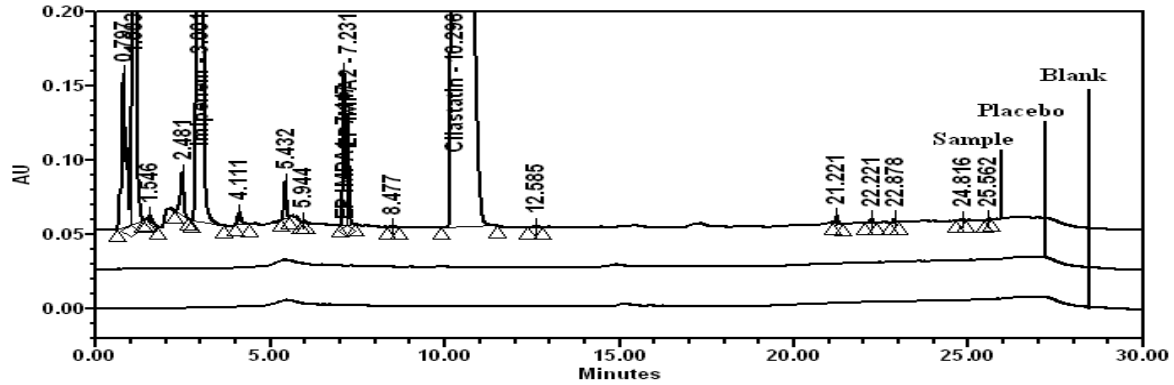


Figure 4.3
Peroxy stress

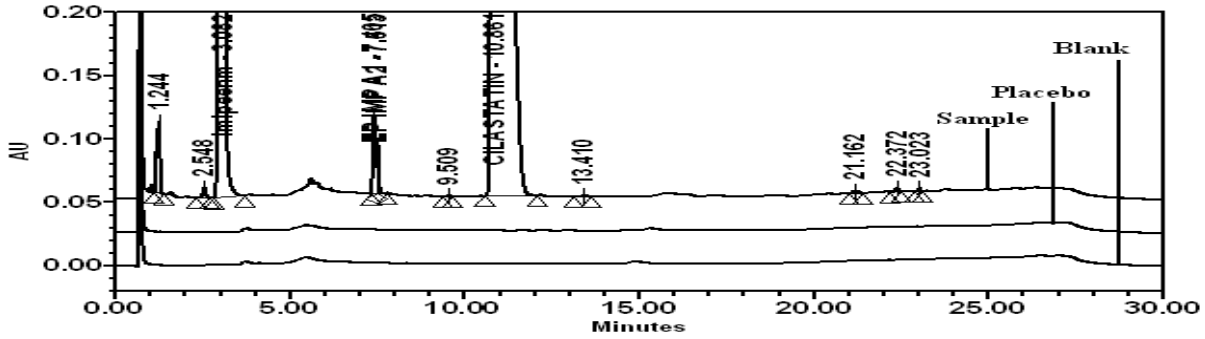


Figure 4.4
Water stress

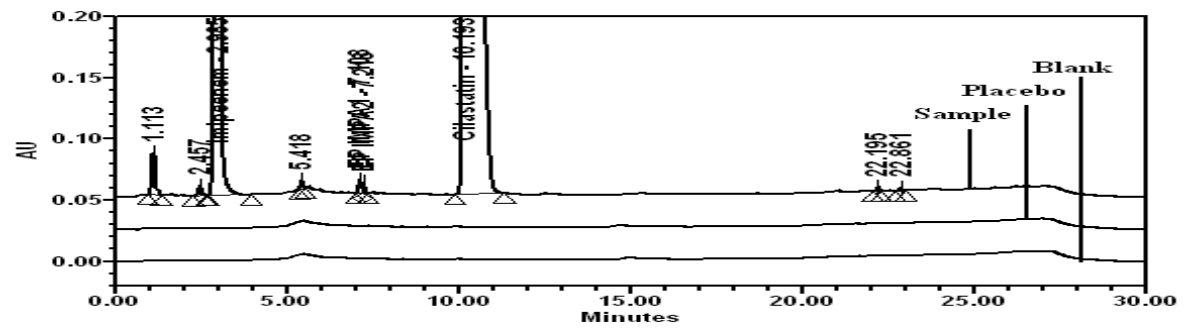


Figure 4.5
Humidity stress

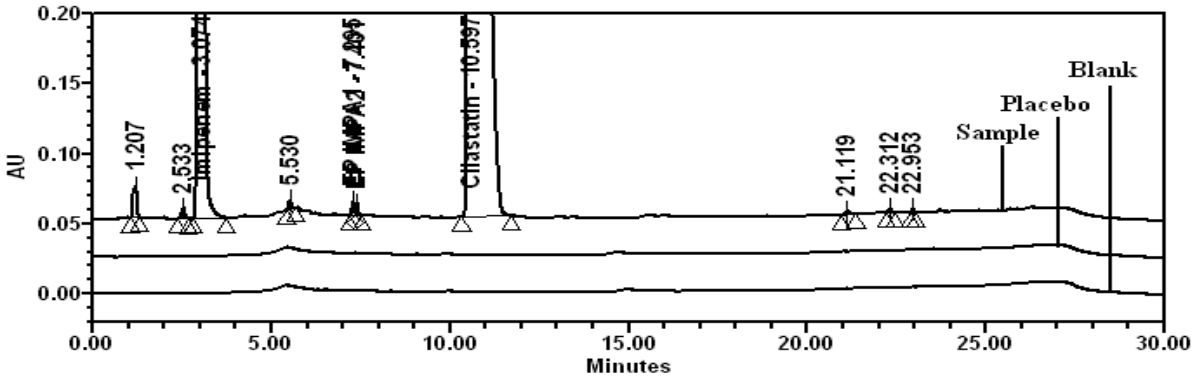
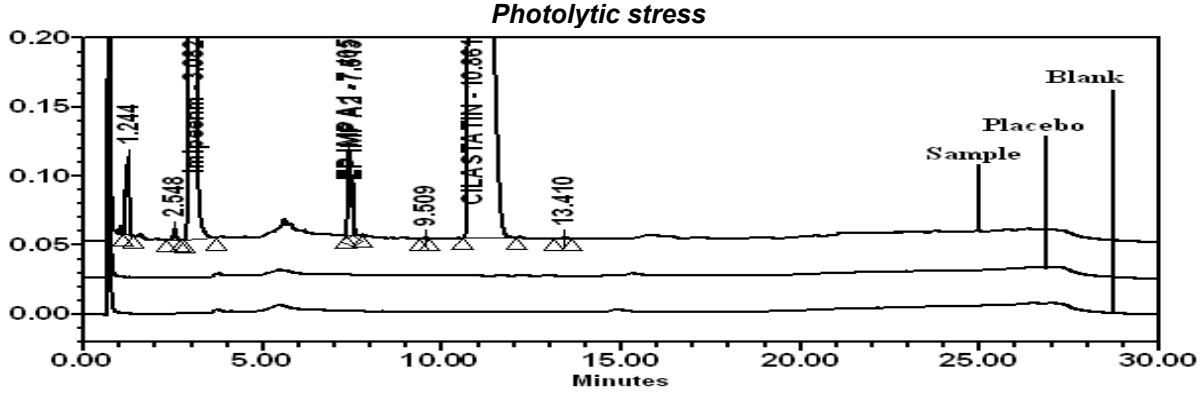


Figure 4.6
Photolytic stress



Peak purity test results from the PDA detector confirmed that the Imipenem & Cilastatin peak obtained from all of the stress samples analysed, was homogenous and pure. Peak purity results from the PDA detector for the peaks produced by the degradation of Imipenem & Cilastatin, confirmed that all these peaks were homogenous and pure for all the stressed samples analysed (Table 3). The mass balance (% assay + % sum of all compounds + % sum of all degradants) results were calculated for all of the stressed samples and were found to be more than 98 % (Table 3). The purity and assay of Imipenem - Cilastatin was unaffected by the presence of its impurities and degradation products, which confirms the stability-indicating power of the developed method.

Condition	%Degradation	Purity Angle		Purity Threshold		Purity Flag		Mass Balance
		IMI	CILA	IMI	CILA	IMI	CILA	
Acid	1.02	0.099	17.642	0.298	28.658	No	No	98.85%
Base	2.09	0.403	27.100	0.593	29.011	No	No	99.65%
Oxidation	0.60	1.702	15.678	3.588	42.804	No	No	100.22%
Water	0.02	1.289	14.137	2.781	39.302	No	No	99.05%
Photolytic	0.10	1.930	16.508	3.700	44.632	No	No	100.34%
Humidity	0.13	2.170	15.000	4.696	41.909	No	No	100.30%

(iii) Results of method validation studies

a. Method validation

The optimized RP-UPLC method validated according to ICH guidelines[11], with respect to specificity, accuracy, precision (repeatability and intermediate precision), linearity, range and robustness. System suitability features were also assessed.

b. Linearity and range

The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of the analyte in the sample. The linearity of the test method was established form the LOQ to 300% of the test concentration for Imipenem & Cilastatin and its related substances. The plots of area under the curve (AUC) of the peak responses of the analytes against their corresponding concentrations, they fitted straight lines responding to equations. The y-intercepts were close to zero with their confidence intervals containing the origin. The correlation coefficient (r) exceeds 0.98, the acceptance threshold suggested for linearity of procedures for the determination of impurity content in bulk drug[12]and it is found to be 0.99 in all the cases. Results were represented in Table 4.

Table 4
Linearity studies of Imipenem-Cilastatin Related substances

Table 4.1
Linearity study for Thienamycin, EP Impurity A1 (EP IMP A1), EP Impurity A2 (EP IMP A2)& In House Impurity 1(IN HOUSE IMP 1)

Recovery level	Thienamycin		EP IMP – A1		EP IMP – A2	
	Added	Rec	Added	Rec	Added	Rec
LOQ	0.2053	0.2032	0.1719	0.1592	0.0948	0.1070
25	1.0135	1.085	0.3943	0.4090	0.3728	0.3585
50	2.0270	1.8132	0.7886	0.7775	0.7457	0.7470
100	4.0541	3.6812	1.5773	1.5745	1.4913	1.5810
150	6.0811	5.7125	2.3659	2.3925	2.2370	2.3642
300	12.1622	12.0232	4.7318	4.8223	4.4739	4.6370

Table 4.2
Linearity study for In House Impurity 2(IN HOUSE IMP 2) EP Impurity C (EP IMP C) & EP Impurity D (EP IMP D)

Recovery level	IN House IMP-1		IN House IMP -2		EP IMP-C		EP IMP-D	
	Added	Rec	Added	Rec	Added	Rec	Added	Rec
LOQ	0.0792	0.0742	0.1273	0.1163	0.0981	0.0922	0.1206	0.1410
25	0.2501	0.2260	0.2702	0.2783	0.5727	0.6193	0.2513	0.2595
50	0.5002	0.4443	0.5403	0.5022	1.2386	1.2578	0.5027	0.5667
100	1.0004	1.0670	1.0806	1.1302	2.4771	2.4403	1.0053	1.0650
150	1.5007	1.6297	1.6210	1.6467	3.7157	4.2320	1.5080	1.5555
300	3.0013	3.0992	3.2419	3.2808	3.2148	3.2435	2.3756	2.3890

c. Determination of limit of quantification and detection (LOQ and LOD)

The linearity performed above, used for the determination of limit of quantification and detection. Residual standard deviation (σ) method was applied and the LOQ and LOD values were predicted using following formulas (a) and (b) and established the precision at these predicted levels. The results are tabulated in Table5.

$$LOQ = \frac{10\sigma}{S} \quad \text{---------(a)}$$

$$LOD = \frac{3.3\sigma}{S} \quad \text{---------(b)}$$

Where σ= residual standard deviation of response and S = slope ofthe calibration curve

Table 5
Limit of quantification & Limit of detection

Name of the impurity	Limit of Quantification			Limit of Detection		
	Conc. µg/mL	% of Impurity	% RSD	Conc. µg/mL	% of Impurity	% RSD
Thienamycin	0.202	0.040	3.2	0.067	0.013	4.1
EP IMP A1	0.181	0.036	2.6	0.060	0.012	4.2
EP IMP A2	0.094	0.019	2.1	0.031	0.006	3.2
*In H Imp-1	0.079	0.016	3.6	0.026	0.005	4.1
*In H Imp-2	0.123	0.025	2.5	0.041	0.008	3.8
EP IMP-C	0.097	0.019	2.4	0.032	0.006	3.4
EP IMP-D	0.118	0.024	2.8	0.039	0.008	3.9

***In house impurity**

d. Accuracy

Accuracy was evaluated by the simultaneous determination of analytes in solution prepared by standard addition method. The experiment was carried out by adding known amount of each related impurities corresponding to different concentration levels of LOQ, 25%, 50%, 100%, 150% and 300% of the specification level in sample solution. The samples were prepared in triplicate at each level. The quantification of added analyte (%weight/weight) was carried out by using an external standard of corresponding main drug prepared at the analytical concentration. The experimental results revealed that approximately 95–105% recoveries were obtained for all the investigated related compounds. Therefore, based on the recovery data (Table 6)the estimation of related compounds that are prescribed in this report has been demonstrated to be accurate for intended purpose and is adequate for routine analysis.

Table 6
Accuracy- Recovery study of Imipenem-Cilastatin Related substances.
Table 6.1
Accuracy- Recovery study of Theinamycin& EP Impurity-A1

% spike Level	Theinamycin			EP Impurity - A1		
	*Added	*Rec	*% Rec	*Added	*Rec	*% Rec
LOQ	0.2053	0.2132	103.8	0.1719	0.1712	99.6
25	1.0135	1.0853	107.1	0.3943	0.4090	103.7
50	2.0270	1.9297	95.2	0.7886	0.7775	98.6
100	4.0541	3.9892	98.4	1.5773	1.5745	99.8
150	6.0811	5.8804	96.7	2.3659	2.3925	101.1
300	12.1622	11.9463	98.2	4.7318	4.8661	102.8

Table 6.2
Accuracy- Recovery study of EP Imp A2 & In House Imp-1

% spike Level	EP Impurity – A2			In House Impurity-1		
	*Added	*Rec	*% Rec	*Added	*Rec	*% Rec
LOQ	0.0948	0.0946	99.8	0.0792	0.0781	98.6
25	0.3728	0.3585	96.2	0.2501	0.2408	96.3
50	0.7457	0.7470	100.2	0.5002	0.4947	98.9
100	1.4913	1.5256	102.3	1.0004	1.0134	101.3
150	2.2370	2.2750	101.7	1.5007	1.4782	98.5
300	4.4739	4.3934	98.2	3.0013	3.0553	101.8

Table 6.3
Accuracy- Recovery study of In House Imp-2 &EP Imp C

% spike Level	In House Imp-2			EP Impurity - C		
	*Added	*Rec	*% Rec	*Added	*Rec	*% Rec
LOQ	0.1273	0.1240	97.4	0.0981	0.0970	98.9
25	0.2702	0.2783	103.0	0.6193	0.6038	97.5
50	0.5403	0.5360	99.2	1.2386	1.2578	101.5
100	1.0806	1.1302	104.6	2.4771	2.4403	98.5
150	1.6210	1.6470	101.6	3.7157	3.6897	99.3
300	3.2419	3.3102	102.1	7.4313	7.5183	101.2

***µg/mL**

e. Method precision

ICH (International Conference on Harmonization of technical Requirements for Registration of Pharmaceuticals for Human Use) considers ruggedness as the method reproducibility and intermediate precision. The data obtained from linearity study was used for the evaluation of method ruggedness. The method reproducibility was determined from the %RSD. The intermediate precision was determined from the difference in the average recoveries and the difference in the %RSD of the recoveries among the three analysts. The RSD of the area of Imipenem & Cilastatin related compounds were within 1.5%. The RSD of results obtained in intermediate precision studies was within 2.5% .The results for all the tested compounds were list edit Table 7 reveal that the method has good reproducibility and intermediate precision.

Table 7
Intraday – Inter day precision studies of Imipenem Cilastatin Related substances

Name of Impurity	Intraday Precision		Interday Precision	
	*% of Impurity	*% RSD	*% of Impurity	*% RSD
Thienamycin	0.8786	1.1	0.8873	2.1
EP IMP A1	0.2849	1.3	0.2960	2.5
EP IMP A2	0.3380	1.4	0.3458	2.4
IN H IMP 1	0.2238	1.3	0.2243	2.3
IN H IMP 2	0.1708	1.2	0.1716	2.5
EP IMP C	0.6769	0.3	0.6832	2.2
EP IMP D	0.2738	1.4	0.2795	1.7

***Mean of six replicates.**

f. 3.3.6. Robustness

In order to demonstrate the robustness of the method, system suitability parameters were verified by making deliberate change in chromatographic conditions, i.e. change in flow rate by±0.1 ml min−1, change in pH of the buffer by ±0.2 units, change in column oven temperature by ±5 ◦C and change in organic composition of mobile phase by ±2% absolute. The sample spiked with all known impurities at impurity tolerance level was injected and there solution among the impurities was monitored. The method was demonstrated to be robust over an acceptable working range of its UPLC operational conditions. The results are tabulated in Table 8.

Table 8
Robustness study of Imipenem Cilastatin Related substances

Parameter		RRT of Impurity						
		Theinamycin	EP Imp A1	EP Imp A2	In House Imp1	In house Imp 2	EP Imp C	EP Imp D
Column Temperature (°C)	35	0.10	0.64	0.65	1.08	1.22	1.86	1.95
	40	0.10	0.64	0.65	1.08	1.21	1.86	1.96
	45	0.10	0.65	0.66	1.09	1.21	1.88	1.97
pH of buffer	2.8	0.10	0.64	0.65	1.08	1.20	1.89	1.99
	3.0	0.10	0.65	0.65	1.08	1.21	1.87	1.96
	3.2	0.12	0.65	0.66	1.08	1.22	1.86	1.95
Flow rate (mL min-1)	0.4	0.10	0.63	0.64	1.07	1.22	1.86	1.96
	0.5	0.10	0.63	0.64	1.08	1.22	1.86	1.97
	0.6	0.11	0.64	0.65	1.08	1.22	1.88	1.96

g. Solution stability and mobile phase stability

The RSD (%) values of all the seven impurities during solution stability and mobile phase stability experiments was within 1.0%. No significant change was observed in the content of impurities during solution stability and mobile phase stability experiments confirm that sample solutions and mobile phase used during the study were stable up to 48 hours.

CONCLUSION

A stability study was carried out and an efficient UPLC method for the quantification of related substances of Imipenem and Cilastatin in drug product was developed and validated. The results of the stress testing of the drug, undertaken according to the ICH guidelines, revealed that the degradation products were formed in hydrolytic(acid and base) conditions. Validation experiments provided proof that the UPLC analytical method is linear in the proposed working range as well as accurate ,precise (repeatability and intermediate precision levels) and specific, being able to separate the main drug from its degradation products. The proposed method was also found to be robust with respect to flow rate, column oven temperature and composition of mobile phase. Due to these characteristics, the method has stability indicating properties being fit for its intended purpose; it may find application for the routine analysis of the related substances of Imipenem and Cilastatin formulations.

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