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Novel RP-HPLC-PDA Approach for Efficient Simultaneous Quantification of Imipenem, Cilastatin and Relebactum in Bulk Drug and Injection Dose Forms

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In this investigation, a highly reliable, precise, stability indicating, specific and selective RP-HPLC approach with photodiode array detection (RP-HPLC-PDA) was established to determine simultaneously imipenem, cilastatin and relebactum in bulk drug and injection dose forms. Chromatographic separation of imipenem, cilastatin and relebactum was achieved *via* using C18 XTerra column and a mobile phase poised of acetonitrile and 0.1 M dipotassium hydrogen phosphate buffer (4.5 pH, set with 0.1% orthophosphoric acid) at 45:55 (v/v) ratio with a flow stream of 1 mL/min. The photodiode array detector was fixed at wavelength 245 nm and quantifications of imipenem, cilastatin and relebactum were based on assessing their peak response areas. Good linearity was detected in target range concentrations of 250-750 µg/mL (imipenem and cilastatin) and 125-375 µg/mL (relebactum). The precision (standard variation percentage) was between 0.141% and 0.257%. Accuracy (%assay nominal) determined was between 99.144% and 99.638%. The validated RP-HPLC approach was applied to Recarbio injection dose evaluating imipenem, cilastatin and relebactum content with no interference encountered from the injection dose inactive ingredients. Imipenem, cilastatin and relebactum were subjected to forced conditions like 30% peroxide, 0.1 N NaOH, sunlight, 0.1 N HCl and 60 ºC. Imipenem, cilastatin and relebactum were effectively separated, quantified and resolved from the degradants generated in forced conditions.

Keywords: Imipenem, Cilastatin, Relebactum, Degradation study, RP-HPLC, Validation.

INTRODUCTION

Imipenem (IPM) is a β-lactam antibiotic classified in the carbapenem subgroup [1,2]. Imipenem is effective towards Gram-positive and also Gram-negative bacteria, both anaerobic and aerobic [2-5]. Penicillin binding proteins are essential for biogenesis of mucopeptides in cell walls of bacteria. Imipenem causes quick bacterial cell death through binding covalently to penicillin binding proteins. Bactericidal effects of imipenem results in inhibition of cell growth, cell proliferation and lack of integrity in cell walls, thus inducing lysis of cell wall.

Cilastatin (CSN) is a chemical substance inhibiting the human dehydropeptidase enzyme [6]. Dehydropeptidase inhabits the renal tubule's brush border and degrades the imipenem antibiotic. In order to safeguard imipenem from dehydropeptidase and lengthen imipenem's antimicrobial property, cilastatin is thus blended intravenously with imipenem. However, cilastatin itself has no antimicrobial property. Proximal tubular necrosis linked with elevated doses of imipenem tends to be avoided by improved renal elimination of unchanged imipenem [7,8].

Relebactam (RBM) is a blocker of β-lactamase known to suppress many categories of β-lactamases, including class A and C enzymes from Ambler [9-11]. Relebactam thus helps avoid the degradation of imipenem in the body and allows imipenem to put forth its bactericidal power for a long time. Relebactam attaches non-covalently to a binding site for β-lactamase, then covalently acylates the amino acid, serine, at the enzyme's active site [12,13].

The combination of imipenem, cilastatin and relebactum was approved in 2019 by the Food and Drug Administration [14]. This combination of imipenem, cilastatin and relebactum is recommended for 18 years of age and older with minimal to no choice for treating complicated infections of urinary tract and intra-abdominal [15-17]. Neither the combination of imipenem, cilastatin and relebactum is not presented in any pharmacopeia nor any methodology has been documented to quantify imipenem, cilastatin and relebactum drugs, simultaneously.

For the first time, herein, a RP-HPLC method coupled with photodiode array detector to quantify imipenem, cilastatin and relebactum simultaneously in bulk drug and injection dose forms is presented.

EXPERIMENTAL

Imipenem, cilastatin and relebactum reference bulk samples were procured from Rainbow Pharma Training Labs (Telangana, India). Recarbio injection dose form (Merck & Co., Inc. USA), claimed to contain 500 mg each of imipenem, cilastatin and 250 mg of relebactum was purchased from the local drug store. Analytical grade dipotassium hydrogen phosphate (S.D. Fine Chem. Ltd., India), hydrochloric acid (Merck, India), sodium hydroxide (S.D. Fine Chem. Ltd., India), orthophosphoric acid (Merck, India) and peroxide (S.D. Fine Chem. Ltd., India), HPLC grade acetonitrile (Merck, India) and Milli Q water (Milli Q system) were employed in this study.

Instrumentation and conditions: The chromatographic separation, detection and quantification of imipenem, cilastatin and relebactum simultaneously in bulk drug and injection dose forms was performed on Waters HPLC system (model number 2695) coupled with photodiodearray detector (model number 2998) employing XTerra C18 (5 µm; 250 mm × 4.6 mm) column. Mobile phase employed consisted of acetonitrile and 0.1M dipotassium hydrogen phosphate buffer (4.5 pH, set with 0.1% orthophosphoric acid) at 45:55 (v/v) ratio. The mobile phase mixture was filtered as well as degassed with a 0.45µ membrane filter prior to use. The same mobile phase mixture was employed as diluent. The optimized values of other parameters include: isocratic method of elution, 1.0 mL/min flow rate, detection of imipenem, cilastatin and relebactum was done using photodiodearray type detector setup at 245 nm, 25 ºC of column temperature and 10 µL of injection volume. The total of 10 min time was needed for single analysis.

Solutions of imipenem, cilastatin and relebactum: Stock combined standard solution of imipenem, cilastatin and relebactum was prepared in mobile phase at a concentration of 5000 µg/mL (IPM), 5000 µg/mL (CSN) and 2500 µg/mL (RBM). Standard combined solution containing imipenem, cilastatin and releb-actum drugs at a concentration of 500 µg/mL (IPM), 500 µg/mL (CSN) and 250 µg/mL (RBM) in mobile phase was prod-uced by diluting aptly the stock combined standard. Further dilutions of stock combined standard were carried out to obtain combined imipenem, cilastatin and relebactum solutions with in target range concentrations of 250-750 µg/mL (IPM), 250-750 µg/mL (CSN) and 125-375 µg/mL (RBM).

Imipenem, cilastatin and relebactum injection dose samples: One Recarbio injection dose form consisting imipenem-500 mg, cilastatin-500 mg and relebactum-125 mg were soaked in 50 mL diluent and was sonicated for solubility for 30 min. It was then filtered and diluted to make up to 100 mL with the similar diluent so that the stock combined injection dose standard of imipenem, cilastatin and relebactum were established at 5000 µg/mL (IPM), 5000 µg/mL (CSN) and 2500 µg/mL (RBM) concentrations. From this stock combined injection dose standard, by proper dilution, a concentration of 500 µg/mL of IPM, 500 µg/mL of CSN and 250 µg/mL of RBM were prepared conferring to its brand claim. The resulting solution was utilized for the estimation of imipenem, cilastatin and relebactum simultaneously in Recarbio injection dose form.

General procedure to assay imipenem, cilastatin and relebactum: Five combined imipenem, cilastatin and relebactum solutions with target range concentrations of 250-750 µg/mL (IPM), 250-750 µg/mL (CSN) and 125-375 µg/mL (RBM) were infused into the HPLC device. To verify whether the instrumental response was absolutely proportional to the analyte's concentration, calibration curves for imipenem, cilastatin and relebactum were established by plotting the analyte's concentration on the X-axis and the analyte's peak area on the Y-axis. Regression equations and values of corelation coefficient for imipenem, cilastatin and relebactum were computed applying linear regression assessment. The content of imipenem, cilastatin and relebactum in unknown samples were evaluated using its related regression equation or calibration curve.

Procedure to assay imipenem, cilastatin and relebactum in Recarbio injection dose form: Test Recarbio injection solution with theoretical concentration of 500 µg/mL of imipenem, 500 µg/mL of cilastatin and 250 µg/mL of relebactum were prepared, then analyzed applying proposed RP-HPLC approach. In the Recarbio test injection solution sample, the amounts of imipenem, cilastatin and relebactum were measured by comparing the peak areas to their respective standard curves or their respective regression equations.

Forced degradation studies: Forced degradation experiments of imipenem, cilastatin and relebactum included stressing the substance in the solution state by using International Council for Harmonization suggested criteria [18]. A stock combined injection dose standard of imipenem, cilastatin and relebactum established in diluent at 5000 µg/mL (IPM), 5000 µg/mL (CSN) and 2500 µg/mL (RBM) concentrations were utilized for forced degradation experiments. These experiments were accomplished by using International Council for Harmonization criteria, under oxidative (30% H_2O_2, sonicated for 30 min), hydrolytic (0.1 N NaOH and 0.1 N HCl, sonicated for 30 min), dry heat (60 ºC, 30 min) and photolytic (sunlight, 6 h) conditions. After degradations, by proper dilution, a threotical concentration of 500 µg/mL of IPM, 500 µg/mL of CSN and 250 µg/mL of RBM was prepared. Applying the proposed RP-HPLC approach, all specimens from forced degradation experiments were investigated.

RESULTS AND DISCUSSION

The purpose of this work was to establish a sensible, stability indicating and effective RP-HPLC with photodiodearray detection system to evaluate imipenem, cilastatin and relebactum simultaneously in bulk drug and injection dose forms. As part of the development work, various stationary phases (Thermo C18, Agilent C18 and Xterra C18) with particle size 5.0 µm were used. Two different mobile phases, like diverse proportions of 0.1 M Na_2HPO_4:methanol and 0.1 M K_2HPO_4:acetonitrile solutions were examined. Better peak shape, peak separation, reasonable retention period (Fig. 1) of imipenem, cilastatin and relebactum were observed on the Xterra C18 (5.0 µm;

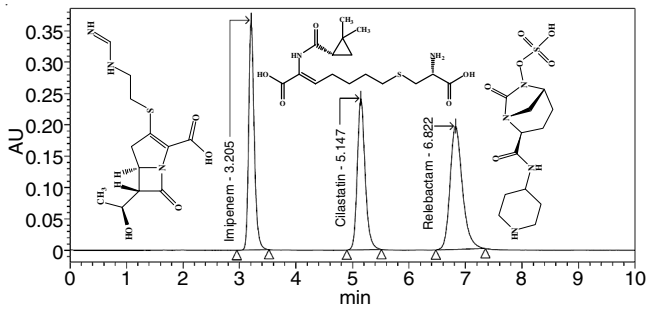

Fig. 1. Chromatograms of imipenem (IPM), cilastatin (CSN) and relebactum (RBM) with their retention times

250 mm × 4.6 mm) column at 25 ºC. The imipenem, cilastatin and relebactum were enumerated using acetonitrile and 0.1M K_2HPO_4 buffer (4.5 pH, set with 0.1% orthophosphoric acid) at 45:55 (v/v) ratio as the mobile phase with 1.7 mL/min flow rate. The imipenem, cilastatin and relebactum were measured with photodiodearray detector setup at 245 nm.

Validation: The RP-HPLC approach mentioned for evaluation of imipenem, cilastatin and relebactum was validated in alignment with criteria of the International Council for Harmonization [19].

System suitability: Standard combined solution (500 µg/mL for IPM, 500 µg/mL for CSN and 250 µg/mL for RBM) was used to evaluate system suitability. The standard combined solution was infused into the system and chromatograms were documented. The theoretical plates number, tailing factor, resolution and standard variation percentage of retention periods and peak areas of three drugs were determined (Table-1). The values obtained for imipenem, cilastatin and relebactum were satisfactory. The device was proved to be suitable for imipenem, cilastatin and relebactum assessment simultaneously.

Selectivity: Blank diluent, standard combined solution (500 µg/mL for IPM, 500 µg/mL for CSN and 250 µg/mL for RBM) and Recarbio injection solution (500 µg/mL for IPM, 500 µg/mL for CSN and 250 µg/mL for RBM) were used for evaluating selectivity. The Xterra C18 column has been infused with these solutions and the respective obtained chromatograms are shown in Fig. 2. Interruption was not spotted at the retention periods of imipenem, cilastatin and relebactum due to blank diluent. For imipenem, cilastatin and relebactum, the retention periods and their elution sequence obtained from the standard combined solution and Recarbio injection solution were matched.

TABLE-1
SYSTEM SUITABILITY OF THE THREE STUDIED DRUGS

Values	RP	RA	RS	PC	TF
Cilastatin					
Mean*	5.152	2352535	9.372	7495.000	1.152
SV	0.006	12415.612	0.038	50.413	0.004
RSV	0.109	0.528	0.409	0.673	0.388
Imipenem					
Mean*	3.208	2506126	–	5902.600	1.212
SV	0.004	16474.354	–	58.218	0.004
RSV	0.120	0.657	–	0.986	0.369
Relebactum					
Mean*	6.835	1205554	5.358	5309.600	1.210
SV	0.011	7305.628	0.027	31.675	0.000
RSV	0.158	0.606	0.501	0.597	0.000

*Mean of five values; SV = Standard variation; RSV = Relative standard variation; RP = Retention period; RA = Response area; RS = Resolution; PC = Plate counts; TF = Tailing factor.

Linearity: Linearity was tested in the 250 µg/mL to 750 µg/mL concentration range for imipenem and cilastatin and from 125 µg/mL to 375 µg/mL concentration range for relebactum. Linear regression data analysis was employed to evaluate the slope, intercept and regression coefficient (Table-2). In the concentration (µg/mL) range evaluated, a value of > 0.99 for regression coefficient presented that the RP-HPLC approach proposed is linear.

Detection limit (LD) and quantification limit (LQ): The values of LQ and LD for imipenem, cilastatin and relebactum, respectively, were measured at signal to noise proportions of 3.3 (LD) and 10 (LQ) (Table-2). The values were proved to be sensitive for imipenem, cilastatin and relebactum assessment simultaneously by the RP-HPLC approach proposed.

Precision and accuracy: The standard combined solution (500 µg/mL for IPM, 500 µg/mL for CSN and 250 µg/mL for RBM) was evaluated as described in the RP-HPLC approach proposed. Accuracy and precision outcomes are outlined in Table-3. Precision values ranged between 0.141% to 0.257% while accuracy values ranged between 99.144% to 99.638%. These findings meet the approval criterion suggested a high reproducibility for imipenem, cilastatin and relebactum determinations simultaneously by RP-HPLC approach proposed.

Recovery: The RP-HPLC approach accuracy was also judged based on the spike recovery of imipenem, cilastatin and relebactum spikes performed at 50% standard level (247.5 µg/mL for IPM, 247.5 µg/mL for CSN and 125 µg/mL for

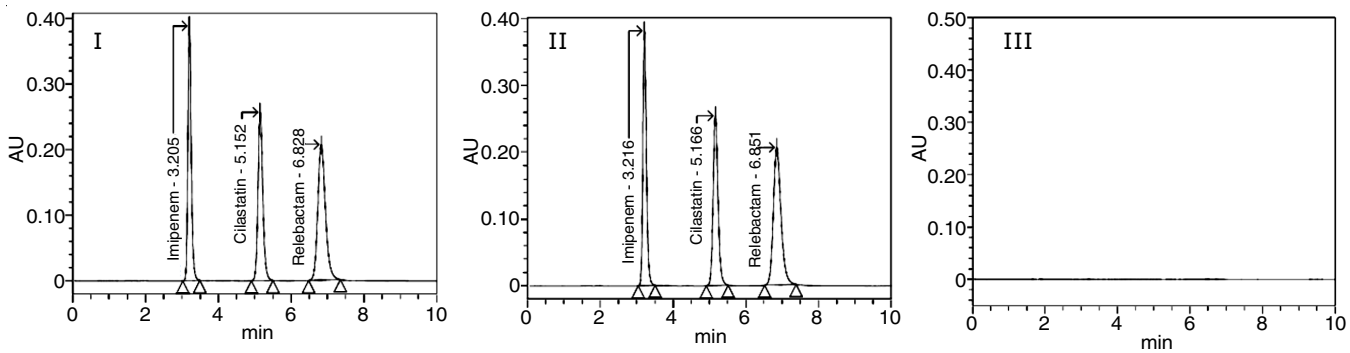

Fig. 2. Chromatograms of (I) standard combined solution; (II) Recarbio injection solution; (III) blank diluent

TABLE-2
REGRESSION EQUATIONS, LINEARITY, LODs AND LOQs OF IMIPENEM, CILASTATIN AND RELEBACTUM

Drug	Linearity (µg/mL)	Regression line equation	Regression coefficient (R^2)	LD	LQ
Imipenem	250-750	y = 5015.9552 x – 21582.4	0.99998	1.281	4.271
Cilastatin	250-750	y = 4703.8624 x – 18033.4	0.99997	1.399	4.666
Relebactum	125-375	y = 4717.3456 x + 13736.8	0.99996	1.195	3.983

y = Peak response area counts; x = concentration of drug (µg/mL)

TABLE-3
PRECISION AND ACCURACY DATA OF IMIPENEM, CILASTATIN AND RELEBACTUM

Drug	Concentration of drug (µg/mL)		Assay* (%)	SV	RSV (%)
	Theoretical	Analyzed*			
Imipenem	500	495.720	99.144	0.140	0.141
Cilastatin	500	496.210	99.242	0.255	0.257
Relebactum	250	249.095	99.638	0.197	0.198

*Mean of six values; SV = Standard variation; RSV = Relative standard variation.

RBM), 100% standard level (495 µg/mL for IPM, 495 µg/mL for CSN and 250 µg/mL for RBM) and 150% standard level (742.5 µg/mL for IPM, 742.5 µg/mL for CSN and 375 µg/mL for RBM) to the Recarbio injection solution (500 µg/mL for IPM, 500 µg/mL for CSN and 250 µg/mL for RBM). The percent spike recovery of imipenem, cilastatin and relebactum was also calculated (Table-4). The accuracy of imipenem, cilastatin and relebactum spiking to the Recarbio injection solution revealed the satisfactory percent recoveries between 99.374-100.091%, 99.484-100.175% and 98.104-99.310%, respectively. These findings also suggesting a high selectivity for imipenem, cilastatin and relebactum determinations simultaneously by RP-HPLC approach proposed in Recarbio injection dose.

Robustness: Robustness of the imipenem, cilastatin and relebactum simultaneous quantitation approach by RP-HPLC was assessed by variation in acetonitrile ratio, column flow, pH, column temperature and wavelength. In brief, the high acetonitrile ratio (50%), low acetonitrile ratio (40%), low column flow (0.9 mL/min), high column flow (1.1 mL/min), high pH (4.7 units), low pH (4.3 units), high column temperature (27 ºC), low column temperature (23 ºC), high wavelength (247 nm) and low wavelength (243 nm) were altered to evaluate the impact on system suitability measures of imipenem, cilastatin and relebactum in a separate run with standard combined solution (500 µg/mL for IPM, 500 µg/mL for CSN and 250 µg/mL for RBM). The theoretical plates number, tailing factor and resolution of three drugs opted were also determined (Table-5). No significant variability in values obtained for the studied drugs. The RP-HPLC approach proposed was proved to be robust after altering vital parameters for imipenem, cilastatin and relebactum simultaneous assessment.

TABLE-4
RECOVERIES FOR THE ASSAY OF IMIPENEM, CILASTATIN AND RELEBACTUM IN RECARBIO INJECTION

Drug	Spike level (%)	Concentration of drug (µg/mL)		Recovered* (%)
		Spiked	Analyzed*	
Imipenem	50	247.5	245.950	99.374
	100	495.0	494.810	99.962
	150	742.5	743.177	100.091
Cilastatin	50	247.5	246.223	99.484
	100	495.0	495.173	100.035
	150	742.5	743.803	100.175
Relebactum	50	125	124.137	99.310
	100	250	245.260	98.104
	150	375	369.450	98.520

*Mean of six values; SV = Standard variation; RSV = Relative standard variation

Specificity and degradation nature of imipenem, cilastatin and relebactum: Table-6 displays the percent degradation and peak purity results of imipenem, cilastatin and relebactum under the forced conditions applied. Based on percent degradation measures, the stability order under the forced conditions applied were as follows:

IPM: Peroxide > sunlight > 0.1N NaOH > 60 ºC > 0.1N HCl

CSN: Peroxide > 0.1N NaOH > sunlight > 0.1N HCl > 60 ºC

RBM: Peroxide > 0.1N NaOH > sunlight > 0.1N HCl > 60 ºC

TABLE-5
ROBUSTNESS STUDIES OF IMIPENEM, CILASTATIN AND RELEBACTUM

Condition		Imipenem			Cilastatin			Relebactum		
		Resolution	Plate counts	Tailing factor	Resolution	Plate counts	Tailing factor	Resolution	Plate counts	Tailing factor
Acetonitrile volume (%)	40	–	5779	1.11	9.07	6729	1.06	5.10	4791	1.15
	50	–	4953	1.19	8.49	5774	1.06	4.81	4066	1.12
Column flow (mL/min)	0.9	–	4933	1.10	8.46	5799	1.05	4.76	4169	1.13
	1.1	–	5779	1.11	9.07	6729	1.06	5.10	4791	1.15
pH (units)	4.7	–	5794	1.22	9.34	7446	1.15	5.37	5287	1.22
	4.3	–	5272	1.26	8.92	6824	1.20	5.15	4930	1.24
Wavelength (nm)	247	–	5439	1.28	8.97	6963	1.21	5.14	4938	1.27
	243	–	5896	1.21	9.35	7479	1.15	5.33	5309	1.21
Column temp. (°C)	23	–	4953	1.09	8.49	5774	1.06	4.81	4066	1.12
	27	–	5572	1.10	8.87	6284	1.06	4.99	4474	1.14

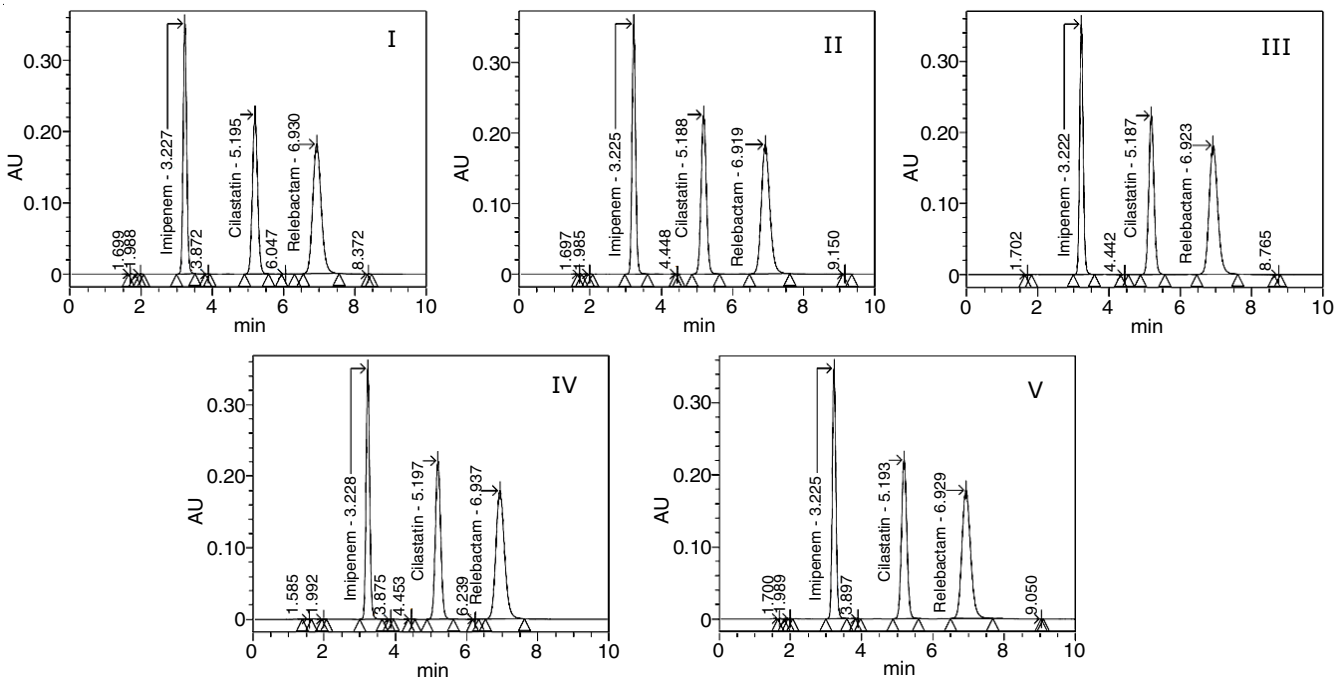

Fig. 3. Chromatograms after exposing of Recarbio injection solution to (I) 0.1 N HCl (II) 0.1 N NaOH (III) 30% peroxide (IV) 60 °C (V) sunlight

TABLE-6
PRECISION AND ACCURACY EVIDENCE DATA OF IMIPENEM, CILASTATIN AND RELEBACTUM

Forced conditions applied	Drug	Degradation (%)	Purity angle	Purity threshold
0.1 N HCl	Imipenem	10.45	0.228	0.551
	Cilastatin	9.82	0.353	0.570
	Relebactum	9.53	0.187	0.440
0.1 N NaOH	Imipenem	7.79	0.240	0.752
	Cilastatin	6.56	0.348	0.671
	Relebactum	6.06	0.188	0.441
30% peroxide	Imipenem	5.53	0.224	0.751
	Cilastatin	4.85	0.356	0.671
	Relebactum	4.10	0.185	0.340
60 °C temp.	Imipenem	10.23	0.233	0.651
	Cilastatin	11.33	0.350	0.771
	Relebactum	10.46	0.184	0.340
Sun light	Imipenem	7.52	0.236	0.652
	Cilastatin	8.28	0.346	0.770
	Relebactum	6.19	0.186	0.440

It has been observed that in 30% peroxide condition, all the three drugs are more stable. Imipenem was more sensitive to 0.1N HCl condition whereas cilastatin and relebactum are more sensitive to 60 ºC condition.

Based on assessment criteria including purity angle and purity threshold employing Waters Empower version 2 tools, the peak purity of imipenem, cilastatin and relebactum was noticed to be homogeneous. Testing of peak purity (purity angle value < purity threshold) demonstrates that perhaps the degradants do not intervene, allowing error-free quantitative analysis of all the studied three drugs simultaneously. The chromatograms obtained under the forced conditions applied on Recarbio injection solution is illustrated in Fig. 3. It was observed from the chromatograms that peaks for degradants were quite well resolved from the studied drugs peaks. Based on these outcomes, the RP-HPLC approach proposed was proved to be specific and stability representing.

Conclusion

In this work, a novel RP-HPLC-PDA method developed for the simultaneous quantification of imipenem, cilastatin and relebactum was found to be a highly reliable, precise, stability-indicating, specific and selective in the bulk drug and injection dosage. The resolution of imipenem, cilastatin and relebactum from the degradation products displays the stability representing characteristics of the RP-HPLC approach proposed.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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