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Research Article

A Novel UPLC Method for the Simultaneous Estimation of Sulbactam and Durlobactam in Pharmaceutical Dosage Form

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ABSTRACT

Infectious diseases especially those associated with multidrug-resistant pathogens in critically ill patients are an area of global research and concern for healthcare professionals across both the private and public sectors. Sulbactam and Durlobactam have been approved as a combination drug, called Xacdura, recently for the treatment of hospital-acquired bacterial pneumonia (HABP) and ventilator-associated bacterial pneumonia (VABP) caused by Acinetobacter baumannii-calcoaceticus complex and is commercially available currently only in the US and China. With 1 million cases of drug-resistant Acinetobacter infections seen globally year-on-year leading to potentially about 300,000 deaths, we expect rapid investments in the development of follow-on drugs, even though the primary patents expire between 2033-2035. Currently, no pharmacopeia method for this combination can aid in quantification or purity testing to aid these pharmaceutical development efforts. Accordingly, we have developed a novel ultra-performance liquid chromatography (UPLC) method for the simultaneous estimation of Sulbactam and Durlobactum. The best chromatographic separation was obtained when the HSSC18 column (1.7µm; 2.1 x 100mm) was used with 0.01N KH2P04: Methanol in 70:30 v/v ratio as mobile phase at an injection volume of 3µL, and run rate of 0.3mL/min. Sulbactam and Durlobactam eluted at 1.823 and 1.345 minutes respectively. The validated method shows high precision, sensitivity, resolution, accuracy, linearity, and robustness as well as reliability and specificity. These findings are expected to accelerate the development and commercialization of the formulations of these drugs with higher efficiency, lower cost, and faster run rate.

INTRODUCTION

Infectious diseases continue to pose significant health challenges by contributing to about 20% of annual deaths globally.[1] While we have made significant strides in antibiotic development and making them accessible globally, antibiotic

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resistance itself has become a pandemic draining healthcare budgets, with high morbidity and mortality, across the world.[2] Among these the multidrug-resistant bacteria continue to be the most critical ones to focus on, especially in the management of critically ill, mostly hospitalized, patients. Acinetobacter, with its ~50 species, poses a huge risk to inpatients and community-setting healthcare management practices with many of them being multidrug-resistant.[3] Acinetobacter baumannii is a difficult-to-treat pathogen and has been labeled as a priority 1 pathogen by WHO, and is a potential cause of 80,000 cases in the US alone annually and about 1 million globally.[4-5] Acinetobacter baumannii is involved in multiple clinical conditions and is known to be the cause behind 100,000-300,000 deaths globally with mortality rates ranging from 8% to 35%.[6-7] Xacduro was approved based on a global phase 3 registrational study, with patients from 16 countries (ATTACK trial), that showed statistical superiority over colistin-treated groups in a 28-day-all-cause mortality as well as statistically significant improvement in overall clinical care.[8] Xacduro is a co-packaged intravenous injection of Sulbactam and Durlobactum and has been recently approved for hospital-acquired bacterial pneumonia (HABP) and ventilator-associated bacterial pneumonia (VABP).[9-10] Sulbactam, a beta-lactam antibacterial inhibits beta-lactamase enzyme thereby preventing the breaking down of beta-lactam antibiotics by drug-resistant bacteria. Sulbactam is most commonly commercialized globally as Ampicillin-Sulbactam and used to treat Acinetobacter-mediated infections.[11] However, there has been an increasing erosion of its antibacterial efficacy leading to higher rates of Sulbactam resistance. Durlobactum is a non-beta-lactam, diazabicyclooctane that aids Sulbactam efficacy by inhibiting other beta-lactamase enzymes, and thus providing a broad spectrum activity against the resistant bacteria as shown in multiple in-vitro, and in-vivo studies.[12-13] The combination has been clinically proven to be efficacious against difficult-to-treat infections caused by Acinetobacter baumannii-calcoaceticus complex. While currently the product is approved only for HABP and VABP, it is expected to confer benefit to patients suffering from other acinetobacter-associated conditions such as complicated urinary tract infections (cUTI), complicated intra-abdominal infections (cIAIs), and sepsis especially not responding to other antibiotics.7,11 Rapid commercialization and uptake of this drug is a crucial unmet need and is expected to be fast-tracked across multiple regulatory agencies by both parent and other pharmaceutical companies. While multiple methods are available for Sulbactam and its combinations, there are currently no pharmacopeia methods available to aid in the testing of Sulbactam and Durlobactam as a combination in their active or pharmaceutical dosage form.[14-15] Accordingly, the objective of this study was to develop an accurate, precise, sensitive, selective, reproducible, and rapid analytical technique for simultaneous estimation of Sulbactam, and Durlobactam in bulk and marketed formulation.

MATERIALS AND METHODS

Chemicals and reagents

Active pharmaceutical ingredients of Sulbactam and Durlobactam pure drugs (Akrivis Pharma Pvt. Ltd.), Combination Sulbactam and Durlobactam injections (Xacduro, 1gm/1gm kit, B and A Pharmaceuticals), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen orthophosphate buffer, Ortho-phosphoric acid were obtained from Rankem. All the methods were conducted in the GLP environment as per the international conference on harmonization (ICH) guidelines (Q2A and Q2B).[16-18]

Instruments

The primary instrument included the ACQUITY UPLC system equipped with quaternary pumps, a TUV detector, and an autosampler integrated with Empower 2 software (Waters). UV-VIS spectrophotometer T60 with special bandwidth of 2mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Sulbactam and Durlobactam solutions (YIS model, PG Instruments). Additionally, basic equipment such as electronic balance (Denvar), pH meter (BVK enterprises, India), and ultrasonicator (BVK enterprises) from the laboratory were utilized in the study.

Solutions and wavelength

Acetonitrile and water were chosen in a 50:50 v/v ratio based on the solubility of the drugs. For the mobile phase, methanol and water were chosen based on the literature analysis. Potassium dihydrogen Orthophosphate (0.01N KH2PO4) was chosen as the buffer. Based on the absorbance spectrum of both drugs Sulbactam and Durlobactam, 248nm was selected as the wavelength for all analytical experiments.

Preparation of standard stock solutions

Sulbactum (10mg) and Durlobactam (5mg) were accurately weighed and transferred into a 10 mL volumetric flask. Three-quarters of the MeCN and water (diluent) were added and the solution was sonicated well. Additional diluent was added and mixed well to obtain a final stock solution containing 1000µg/mL of Sulbactam and 500µg/mL of Durlobactam. A standard working solution was made by aliquoting 1mL of the above standard stock solution into a 10mL volumetric flask and diluting the same with the diluent to obtain a solution containing 100µg/mL of Sulbactam and 50µg/mL Durlobactam.

Preparation of sample stock and working solutions

One vial (equivalent to 1000 mg of Sulbactam and 500mg of Durlobactam) was taken into a 500 mL volumetric flask and 50 mL of Acetonitrile and water (diluent) was added, shaken for 5 minutes manually, and further sonicated for 5 minutes. The solution was diluted up to 500mL mark with diluent to form the sample stock solution. For the preparation of the working sample solution, 0.5 mL of this solution was further transferred into a 10 mL of volumetric flask and diluted up to 10 mL with the diluent and mixed well. The solution was filtered through a 0.2µm nylon membrane filter. From the filtered solution 1 ml was pipetted out into a 10 mL volumetric flask and made up to 10mL with diluent.

Preparation of the buffer

To prepare 0.01N Potassium dihydrogen Orthophosphate (KH2PO4) buffer, 1.36g of KH2PO4 was taken in a 1000mL volumetric flask, and 900mL of the milli-Q water was added to sonicate well. The volume was finally made up to 1000mL and the solution was titrated to pH 3.5 using a dilute Orthophosphoric acid solution.

System suitability parameters

The system suitability parameters were determined by preparing standard solutions of Sulbactam (100 ppm) and Durlobactam (50 ppm). Parameters like peak tailing, resolution, and USP plate count were determined from the data obtained by injecting the solution six times. The % RSD for the area of six standard injections results were calculated and compared against the recommended specifications (%RSD<2%).

Specificity

To assess the specificity of the assay, the injections of the blank, as well as placebo samples, were compared with the injections of the standard solutions containing Sulbactam and Durlobactam. The absence of interfering peaks in blank and placebo at retention times of these drugs would indicate that the optimized method is specific.

Precision

The standard stock as well as the working solutions were used to assess the interday and intraday precision of the method. Each of the samples was injected in triplicates and a %RSD value of <2 is considered to be an indicator of method precision.

Linearity

To assess the linearity of the assay standard calibration curves were evaluated. The range of the analytical method was established by taking the lower concentration to higher concentration intervals using the regression coefficient equation of linear plots. To conduct the linearity experiments, 6 working solutions encompassing different concentrations ranging from 25 to 150µg/mL of Sulbactam and 12.5 to 75µg/mL of Durlobactam respectively were made from 25% to 150% of the standard stock solutions of Sulbactam and Durlobactam.

Accuracy

The accuracy of the assay was measured by the standard addition method at three different concentration levels (50, 100, and 150%). A standard stock solution containing 100µg/mL of Sulbactam and 50µg/mL of Durlobactam was diluted to form 10mL spiked solutions by adding 25µL, 50µL, and 75µL of Durlobactam, and 50µL, 100µL, and 150µL of Sulbactam sample stock solution respectively for yielding 50%, 100%, and 150% levels. The experiment was done as triplicates and mean values were used. The % Recoveries were calculated by applying the regression equation. The % Recovery for each level between 98 to 102 was considered an indicator of accuracy.

Robustness

Small deliberate changes in defined system suitability parameters like flow rate (Flow minus: 0.2ml/min, Flow plus: 0.4mL/min), mobile phase ratio (mobile minus 55B:45A, mobile plus 65B:35A), and temperature (minus: 25°C; plus: 35°C) were made both on the lower and upper end and the samples analyzed by injecting in duplicates. Obtaining the results to be within range as per ICH guidelines (%RSD was within the limit) was considered an indicator of assay robustness.

Degradation studies

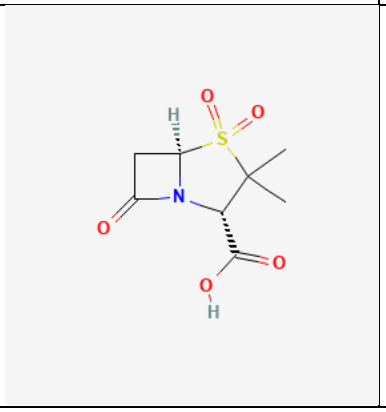
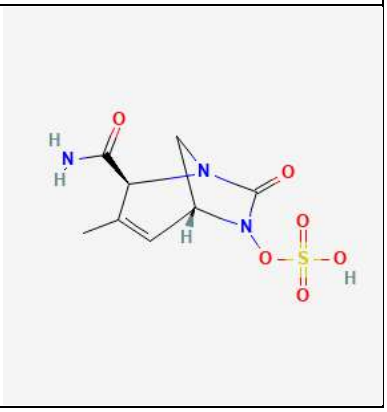
Degradation studies were conducted across 6 media, acidic, basic, neutral, oxidation, thermal, and photo/UV. As a first step, 1mL each of the Sulbactam and Durlobactam stock solutions was mixed with 1mL of the degradant solution for oxidation, acid, and base degradation studies. For the oxidation study, one mL of 10% hydrogen peroxide (H2O2) was added to 1 mL of each of the Sulbactam and Durlobactam stock solutions. Similarly, for the acid and alkali degradation study, 1mL of 1N hydrochloric acid and IN sodium hydroxide respectively were used. For each of the degradation studies, the drug and degradation medium solutions were kept for 30 min at 600c. For the dry heat degradation solution, the standard drug solutions were placed in the oven for an hour at 105°C. The photochemical stability of the drug was studied by exposing the 1000µg/mL Sulbactam and 500µg/mL Durlobactam solution to UV light by keeping the beaker in the UV chamber for one day through exposure to 200-Watt hours/m2 in the photostability chamber is also equally acceptable. Stress testing under neutral conditions was studied by refluxing the drug in water for 1 hour at a temperature of 60ºC. For UPLC, the resultant solution, post degradation period, was diluted to obtain 100µg/mL and 50µg/mL solution of the Sulbactam and Durlobactam respectively and 0.30 µL was injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSION

Literature analysis indicated that while UPLC is the most preferred method, it has not been

developed so far to identify Sulbactam and Durlobactam simultaneously. For a better understanding of the potential methods and the associated parameters, a detailed literature analysis on both drugs was undertaken. Sulbactam with an IUPAC name of (2S,5R)-3,3-dimethyl-4,4,7-trioxo-4λ6-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid has a molecular formula and weight of C8H11NO5S and 233.24 g/mol respectively while Durlobactam is [(2S,5R)-2-carbamoyl-3-methyl-7-oxo-1,6-diazabicyclo[3.2.1]oct-3-en-6-yl] hydrogen sulfate with a molecular formula and weight of C8H11N3O6S and 277.26 g/mol respectively.19-20 The physical and chemical parameters of both drugs are described in Table 1.

Table 1. Physical and Chemical Characteristics of Sulbactam and Durlobactam

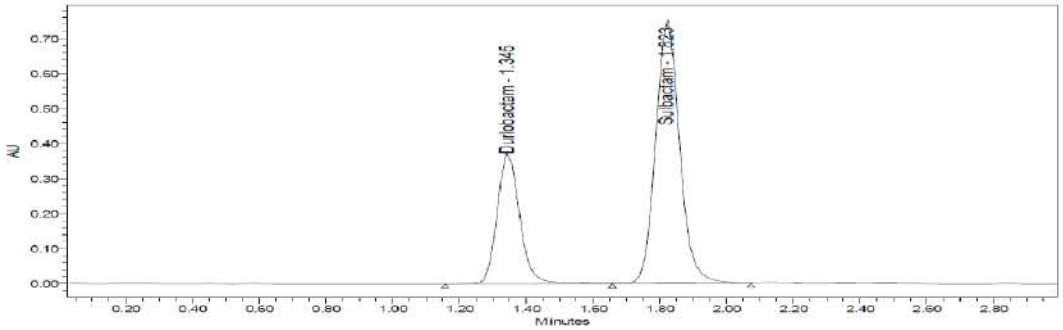
	Sulbactam	Durlobactam
CAS Number	68373-14-8	1467829-71-5
Molecular Weight	233.24	299.33
Molecular Formula	$C_8H_{11}NO_5S$	$C_8H_{10}N_3NaO_6S$
Physical State	Solid	Solid
Solubility	In water (47 mg/ml at 25° C), DMSO (47 mg/ml at 25° C), and ethanol (47 mg/ml at 25° C).	In water (6.5 mg/ml at 25° C)
pKa	3.09	-1.9
Published Analytical Methods	RP-HPLC and UPLC	None
Structure		

The standard solution of both Sulbactam and Durlobactam showed an absorbance at 248 nm and hence the analytical wavelength of 248 nm was chosen for method development and optimizations. The focus of our method development was to modify various mobile phases and buffer ratios. The optimization parameters used across the trials with each modification and the associated results are shown in Table 2. As can be seen, the HSS C18 column was better than the CHS C18 column as the USP plate count resolution improved more than 2000 with the same. The mobile phase composition ratio was slowly changed and eventually 0.01N KH2PO4 and Methanol in 70: 30 v/v ratio yielded symmetric and resolved peaks. Durlobactam was eluted at 1.345 minutes while the Sulbactam peak was observed at 1.823 min. Plate count and tailing factors were also satisfactory and hence these conditions were chosen as the optimized method for further validation. (Table 2, Column 5, Figure 1).

Table 2. Parameters Tested for Development of the Optimal UPLC Method

	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Mobile Phase	0.01N KH_2PO_4 : Methanol				
Mobile Phase Ratios	50:50 v/v	50:50 v/v	55:45 v/v	60:40 v/v	70:30 v/v
Flow rate	0.3 mL/min	0.3 mL/min	0.3 mL/min	0.3 mL/min	0.3 mL/min
Column	CHS C_{18}	HSS C_{18}	HSS C_{18}	HSS C_{18}	HSS C_{18}
Column Dimensions	2.1 x 100mm, 1.7µm				
Detector wavelength	248nm	248nm	248nm	248nm	248nm
Column temperature	26.0°C	26.0°C	26.0°C	26.0°C	26.0°C
Injection volume	3.0µL	3.0µL	3.0µL	3.0µL	3.0µL
Run time (min)	10.0 min	10.0 min	10.0 min	10.0 min	10.0 min
Diluent	Methanol and Water				
Diluent's Ratio	50:50 v/v	50:50 v/v	50:50 v/v	50:50 v/v	50:50 v/v
Elution	OK	OK	OK	OK	OK
Peak	Asymmetric	Merged	Merged	Merged	Symmetric
Resolution	>2	<2	<2	<2	Resolved

Figure 1. Optimized Chromatogram of Sulbactam and Durlobactam



The LOD and LOQ of the assay for Sulbactam were 0.19µg/mL and 0.56µg/mL respectively and 0.08µg/mL and 0.026µg/mL respectively for Durlobactam. The chromatogram data with 6 injections at the optimized conditions was compiled and the critical system suitability parameters for Sulbactam and Durlobactam against the recommended parameters were evaluated and have been listed in Table 3. As can be seen, all the parameters have passed and are as per ICH stipulated guidelines.

Table 3. System Suitability Parameters and Their Recommended Limits: Data from Sulbactam and Durlobactam Assay

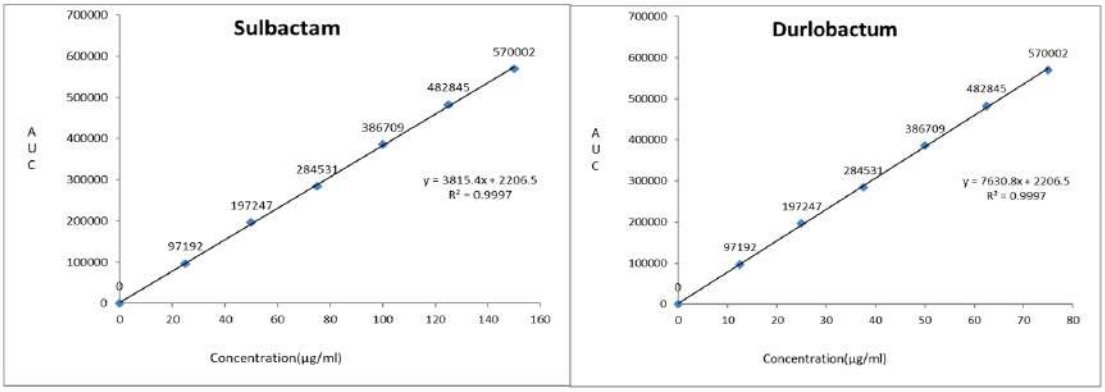
Parameter	Recommendation	Sulbactam	Durlobactam
Capacity Factor (K')	Void volume K>2 and K' > 2	>2	>2
Repeatability	RSD ≤ 2% N ≥ 5 is desirable	6	6
Relative Retention	Not essential as the resolution is stated	NA	NA
Resolution(Rs)	Rs of > 2 between the peak of interest and the closest eluting potential interferent	>2	>2
Tailing Factor(T)	T ≤ 2	1.123	1.143
Theoretical Plates(N)	> 2000	3188	2984

Retention times of Sulbactam and Durlobactam were 1.823 and 1.345 minutes respectively. We did not find any interfering peaks in blank and placebo at retention times of these drugs in this method (data not shown) and hence believe the optimized method is specific to simultaneous detection and quantification of Sulbactam and Durlobactam. The calibration curve for Sulbactam and Durlobactam was run from 25% to 150% level (25, 50, 75, 100, 125, and 150 µg/mL) and 12.5 to 75 µg/mL (12.5, 25, 37.5, 50, 62.5 and 75 µg/mL) respectively. All the injections were run in duplicate and the average areas were used to plot the linearity curve with area under the curve of the y-axis and tested concentrations on the x-axis. The linearity equation of y = 3815.4x + 2206.5 and y = 3555.8x + 1743 for Sulbactam and Durlobactam was obtained with a correlation coefficient of 0.999 for both, in the regression analysis, indicating clear linearity of the method for assessing Sulbactam and Durlobactam in the 25 to 150 µg/mL and 12.5 to 75 µg/mL concentration range. (Table 4, Figure 2).

Table 4: Linearity Table for Sulbactam and Durlobactam

Sulbactam		Durlobactam	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
25	97192	12.5	45584
50	197247	25	91842
75	284531	37.5	134940
100	386709	50	184922
125	482845	62.5	222332
150	570002	75	265991

Figure 2. Calibration Curves of Sulbactam (2a) and Durlobactam (2b) Linearity



The method was validated according to the ICH guidelines and as specified in Table 3. When injections were given from the same standard solution flask of both Sulbactam and Durlobactam and areas obtained, it was found that %RSD was 0.5% and 1.0% respectively, both of which are below 2 indicating that the established method is precise. Six working sample solutions of the same concentrations were prepared from one sample stock solution, injected into the columns and areas were obtained as provided in Table 5. Average area, standard deviation, and % RSD were calculated for two drugs and obtained as 0.5% and 1.0% respectively for Sulbactam and Durlobactam. When the same solutions were injected the next day and the areas obtained, we observed that the calculated %RSD was 0.6% and 0.4% respectively (Table 5 Day 2). With the limit of precision less than 2 in both intraday (Table 5- Day 1a and Day 1b) and interday (Table 5- Day 1 (a and b) v/s Day 2) the system is precise as it has passed the test per ICH guidelines.

Table 5. Precision Data (Interday and Intraday) for Durlobactam and Sulbactam

	Day 1a		Day 1b		Day 2	
S. No	Durlobactam	Sulbactam	Durlobactam	Sulbactam	Durlobactam	Sulbactam
1	181805	387596	182415	388958	178184	367296
2	182401	395123	183523	390055	179625	370517
3	183179	387381	182909	391382	178835	370171
4	183526	392591	185657	390337	179153	369056
5	184338	386471	186353	386175	177849	368545
6	183493	387284	182075	386823	178642	373569
Mean	183124	389408	183822	388955	178715	369859
S.D	898.6	3558.7	1773.6	2063.2	643.7	2155.7
%RSD	0.5	0.9	1	0.5	0.4	0.6

The accuracy of the established method was evaluated at three different levels using standard addition methods (50%, 100%, and 150%). The study was conducted by injecting samples from each level in triplicate and assessing the mean % RSD values. The % RSD was found to be within acceptable limits for both the drugs across all three levels indicating the method is accurate (Table 6)

Table 6. Accuracy Data of Sulbactam and Durlobactam

	Sulbactam					Durlobactam				
% Level	Amount Spiked µg/mL	Amount recovered µg/mL	% Recovery	% Mean RSD	SD	Amount Spiked µg/mL	Amount recovered µg/mL	% Recovery	% Mean RSD	SD
50%	50	49.70	99.40	99.71	0.59	25	25.29	101.18	100.03	1.02
	50	49.67	99.35			25	24.93	99.71		
	50	50.20	100.39			25	24.8	99.21		
100 %	100	99.92	99.92	99.79	0.21	50	50.73	101.46	100.28	1.02
	100	99.56	99.56			50	49.87	99.74		
	100	99.91	99.91			50	49.82	99.64		
150 %	150	148.29	98.86	98.95	0.09	75	73.89	98.52	99.38	0.81
	150	148.45	98.97			75	75.1	100.13		
	150	148.55	99.04			75	74.64	99.51		

The robustness of the assay was validated by changing select parameters, in both directions, such as flow rate (flow plus 0.2mL/min and flow minus 0.3mL/min), mobile phase (mobile phase plus 65B:35A and mobile minus 55B:45A), and temperature (plus 35°C and minus 25°C). All the injections were carried out in duplicates and the mean data was evaluated against the optimized methods systems parameters. It was observed that system suitability parameters did not get affected by the tested variances and all of them were per the specifications i.e., % RSD was within the stipulated limits (<2) across all parameters. (Table 7).

Table 7. Robustness of the Assay for Sulbactam and Durlobactam

S.No	Parameter	Condition	%RSD of Sulbactam	%RSD of Durlobactam
1	Change in Flow Rate (mL/min)	Flow rate (-) 0.7ml/min	0.1	0.9
2		Flow rate (+) 0.9ml/min	0.2	0.3
3	Change in Mobile Phase	Mobile phase (-) 50B:50A	0.6	0.7
4		Mobile phase (+) 60B:40A	0.8	0.4
5	Change in Temperature	Temperature (-) 25°C	0.9	0.1
6		Temperature (+) 35°C	0.8	0.2

The assay was performed with a formulation containing 1000mg of Sulbactam and 500mg of Durlobactam. The average % assay for Sulbactam and Durlobactam was 99.79% and 99.98% respectively (Table 8). Degradation studies were performed with the combination drug formulation across different conditions (acid, basic, neutral, thermal, UV, and oxidation states), and the degraded samples from each condition were injected into the columns. The assay of the injected samples was calculated and all the samples passed the limits of degradation as indicated in Table 9.

Table 8. Assay Data of Sulbactam and Durlobactam

	Sulbactam			Durlobactam		
S.No	Standard Area	Sample area	% Assay	Standard Area	Sample area	% Assay
1	387596	388958	99.68	181805	182415	99.21
2	395123	390055	99.97	182401	183523	99.82
3	387381	391382	100.31	183179	182909	99.48
4	392591	390337	100.04	183526	185657	100.98
5	386471	386175	98.97	184338	186353	101.36
6	387284	386823	99.14	183493	182075	99.03
Avg	389408	388955	99.79	183124	183822	99.98
Stdev	3558.7	2063.2	0.529	898.6	1773.6	0.965
%RSD	0.9	0.5	0.5	0.5	1	0.96

Table 9. Degradation Data of Sulbactam and Durlobactam

S.No	Degradation Condition	% Sulbactam Degraded	% Durlobactam Degraded
1	Acid	5.7	4.1
2	Alkali	7.57	5.88
3	Oxidation	5.82	4.25
4	Thermal	2.86	2.19
5	UV	1.98	1.79
6	Water	0.96	0.8

CONCLUSION

A simple, accurate, and precise method was developed for the simultaneous estimation of the Sulbactam and Durlobactam in injection form. The retention time of Sulbactam and Durlobactam were found to be 1.823 min. and 1.345 min while the % RSD of the Sulbactam and Durlobactam were found to be 0.8 and 0.6 respectively. The recovery percentage in the assay was 99.49% and 99.90% for Sulbactam and Durlobactam respectively. LOD and LOQ values obtained from regression equations of Sulbactam and Durlobactam were 0.19, 0.56 µg/mL and 0.08, 0.26 µg/mL respectively. The Regression equation of Sulbactam was defined as y = 3815.4x + 2206.5, and y = 3555.8x + 1743, of Durlobactam. The established method with the validation data obtained in this study combined with the fast run rate makes it a first and critical UPLC method that can accelerate the access of the combination drug to patients across the globe.

CONFLICT OF INTEREST

None of the authors have any conflict of interest.

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