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"DEVELOPMENT AND VALIDATION OF A ROBUST RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF SULBACTAM AND DURLOBACTAM IN BULK AND TABLET DOSAGE FORMS"

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ABSTRACT

A simple, precise, and reliable method was developed for the simultaneous estimation of Sulbactam and Durlobactam in tablet dosage forms. Chromatographic separation was performed using an Agilent C18 column (250 × 4.6 mm, 5 µm) with a mobile phase consisting of 0.1% orthophosphoric acid and acetonitrile in a 70:30 ratio, delivered at a flow rate of 1.0 mL/min. The column temperature was maintained at 30°C, and detection was carried out at a wavelength of 220 nm. The retention times for Sulbactam and Durlobactam were observed at 2.458 min and 2.855 min, respectively. The method demonstrated excellent precision, with %RSD values of 0.3 for Sulbactam and 0.6 for Durlobactam, while the recovery rates were 99.29% and 99.87%, respectively. Limits of detection (LOD) and quantitation (LOQ) were calculated from the regression equations, yielding 0.09 and 0.27 for Sulbactam, and 0.04 and 0.13 for Durlobactam. The regression equations obtained were y = 2684.5x + 484.45 for Sulbactam and y = 2153.5x + 150.3 for Durlobactam. Overall, the method offers reduced retention times and shorter run durations, making it a cost-effective and practical approach suitable for routine quality control analysis in the pharmaceutical industry.

KEYWORDS: Sulbactam, Durlobactam, RP-HPLC

1.1 INTRODUCTION

The quality of any pharmaceutical product, which in turn determines its safety and clinical efficacy, is crucial to its therapeutic success. Systems for pharmaceutical quality assurance

are designed to ensure that drug ingredients and finished dosage forms consistently meet predetermined requirements throughout their lifecycle. Pharmaceutical analysis is a key component of this framework since it offers scientific proof that a product is suitable for patient usage. Analytical technique development is a crucial regulatory requirement since regulatory bodies are increasingly emphasizing that analytical results are only as reliable as the methods used to create them[3,4].

Assay and impurity control are the two main criteria used in the quality assessment of pharmaceutical products. While impurity analysis deals with degradation products, residual solvents, and contaminants linked to the process or formulation, assay determination verifies that the active pharmaceutical ingredient (API) is present within permissible limits to assure therapeutic efficacy[6]. Sensitive and selective analytical methods are crucial because some contaminants may be harmful even at extremely low quantities. Reduced efficacy, unfavorable medication responses, or regulatory rejection can arise from inadequate assay or impurity control[7].

The wide range of physicochemical properties of drug molecules, including polarity, solubility, molecular size, chemical stability, and functional group composition, makes it difficult to develop analytical techniques appropriate for pharmaceutical applications. Methods for routine quality control must be quick, affordable, accurate, repeatable, and simple to use in addition to having high analytical performance [8,9,10]. The adoption of cutting-edge experimental procedures and ongoing improvement of current analytical techniques have been fueled by the need to balance these demands.

Numerous chemical and physicochemical methods are used in pharmaceutical analysis. Because they are straightforward and reliable, traditional analytical techniques like titrimetric and gravimetric procedures are still useful. Nuclear magnetic resonance (NMR), mass spectrometry (MS), UV–visible, infrared, fluorescence, and other spectroscopic methods offer important qualitative and quantitative data. However, due to their exceptional capacity to separate and quantify numerous components in complicated matrices, chromatographic techniques have become essential [11,12].

A collection of separation methods based on the distinct distribution of analytes between a stationary phase and a mobile phase is referred to as chromatography. Effective separation is made possible by the different retention behaviors that result from different molecular

interactions with these phases. Chromatographic techniques can be categorized based on the major separation mechanism, the stationary phase's nature, or the mobile phase's physical state. Analytical chromatography is widely utilized in pharmaceutical research and quality control for identification confirmation, purity assessment, and quantitative determination, whereas preparative chromatography is mostly employed for purification and isolation.[10,11,12].

1.2 Classification of HPLC

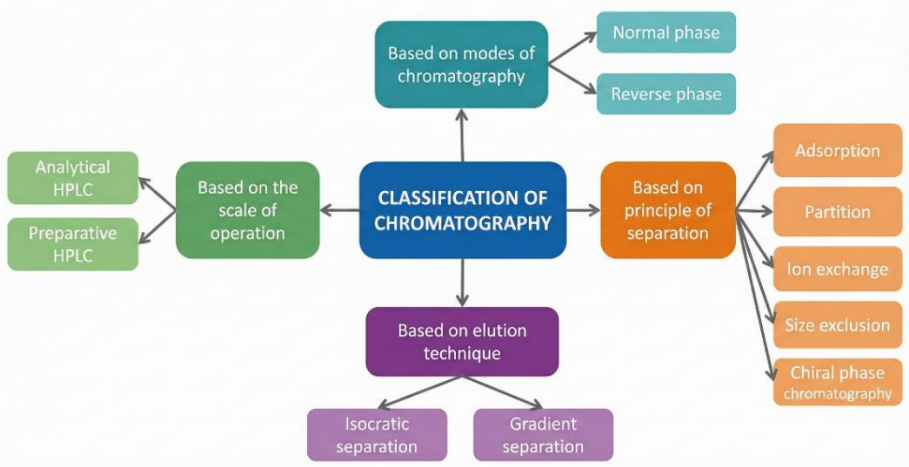


Figure 1:Figure X. Overview of Chromatography Classification.

In pharmaceutical laboratories, high-performance liquid chromatography (HPLC) is the most popular analytical instrument among chromatographic techniques. HPLC is appropriate for routine analysis of APIs, contaminants, and completed dosage forms due to its excellent resolution, sensitivity, precision, and reproducibility[13]. Chromatographic efficiency has been greatly increased while analysis time has decreased thanks to technological developments in detector design, high-pressure pumping systems, and column packing materials. A variety of pharmaceutical chemicals can be analyzed using a variety of HPLC modes, with reversed-phase HPLC being the most widely used. These modes include normal-phase, reversed-phase, ion-exchange, size-exclusion, affinity, and chiral chromatography.[14,15].

Appropriate selection and optimization of chromatographic conditions, including stationary phase, mobile phase composition, flow rate, and detection parameters, are critical to the dependability of an HPLC process. System suitability tests are carried out to confirm chromatographic performance in terms of retention, resolution, efficiency, and peak symmetry prior to normal application. When examining low-level contaminants and degradation products, these metrics are especially important [14,16].

1.3 System Suitability Parameters in HPLC (Conceptual Flow)

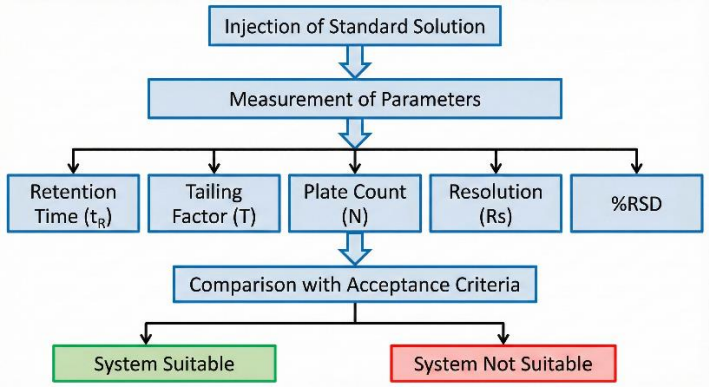


Figure 2:System Suitability Parameters in HPLC (Conceptual Flow)

1.4 Regulatory Perspective and ICH Alignment

International regulatory criteria must be followed by analytical techniques used for pharmaceutical quality control. Analytical procedure validation requirements are defined by the International Council for Harmonization (ICH) rules, namely ICH Q2(R1) and the modified ICH Q2(R2). Validation features like specificity, linearity, accuracy, precision, detection limit, quantitation limit, robustness, and range are all specified in these guidelines. In order to support regulatory submissions and guarantee consistent product quality, compliance with ICH Q2 guarantees that analytical techniques are reliable, repeatable, and appropriate for their intended use. [8.,9,2].

2 Objective of the Study

To develop and validate a rapid, accurate, precise, sensitive, and stability-indicating HPLC method for the simultaneous estimation of Sulbactam and Durlobactam in bulk drug and tablet dosage forms in accordance with ICH guidelines.

3 Materials

Sulbactam and Durlobactam pure drugs (API), Sulbactam and Durlobactam (**Xacduro**) Injection, Distilled water, Acetonitrile, Phosphate buffer, , Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem

4 Instruments:

- Electronics Balance-Denver
- p^H meter -BVK enterprises, India
- Ultrasonicator-BVK enterprises
- WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software.
- UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Sulbactam and Durlobactam solutions.

5 Methodology

5.1 Preparation of Solutions

a. Diluent

For the production of standard and sample solutions, a 50:50 (v/v) mixture of acetonitrile and water was chosen as the diluent based on the solubility characteristics of Sulbactam and Durlobactam[16,17,18].

b. Preparation of Standard Stock Solution

Sulbactam (20 mg) and Durlobactam (10 mg) were precisely weighed and put into different 50 mL volumetric flasks. After adding around three-fourths of the diluent, the solutions were sonicated for ten minutes to guarantee total dissolution. After that, the quantities were adjusted with diluent to produce standard stock solutions with 200 µg/mL of durlobactam and 400 µg/mL of sulbactam. Sonication is frequently used to increase solution homogeneity and solubility.[19,20].

c. Preparation of Standard Working Solution (100%)

To create working standard solutions with 40 µg/mL of Sulbactam and 20 µg/mL of Durlobactam[21], 1.0 mL of each standard stock solution was put into a 10 mL volumetric flask and diluted to volume with diluent.

d. Preparation of Sample Solutions

- **Sample Stock Solution**

A 500 mL volumetric flask was filled with the contents of a single-dose vial containing 500 mg of durlobactam and 1000 mg of sulbactam. After adding around 5 mL of diluent, the mixture was sonicated for 25 minutes. After adding diluent to the volume, it was filtered through a 0.45 µm HPLC filter to create a sample stock solution with 2000 µg/mL of sulbactam and 1000 µg/mL of durlobactam [22,23,24].

- **Sample Working Solution (100%)**

40 µg/mL of Sulbactam and 20 µg/mL of Durlobactam were obtained by diluting an aliquot of 0.2 mL of the filtered sample stock solution to volume with diluent in a 10 mL volumetric flask.

e. Buffer Preparation

- **0.1% OPA Buffer:**

One milliliter of concentrated orthophosphoric acid was diluted to 1000 mL with purified water[25].

- **N Potassium Dihydrogen Phosphate Buffer:**

A precisely weighed 1.36 g of potassium dihydrogen orthophosphate was dissolved in around 900 mL of Milli-Q water, degassed by sonication, and then diluted to 1000 mL. After adding 1 mL of triethylamine, diluted orthophosphoric acid was used to bring the pH down to 3.5.[26,27,28].

5.2 Method Validation

a. HPLC Method Development and Validation

The creation of high-performance liquid chromatography (HPLC) methods is a methodical procedure intended to accomplish accurate pharmaceutical analyte separation, identification, and quantification. To find appropriate chromatographic techniques and reduce experimental effort, the procedure starts with a review of the body of current literature. Analyte physicochemical characteristics such polarity, ionization, molecular size, and stability serve as a guidance for method selection [29, 30, 31.] To guarantee system compatibility and improve detectability, sample preparation—including dissolving, filtration, extraction, cleanup, or derivatization—is carried out. Normal-phase, ion-exchange, and size-exclusion methods are chosen for certain analytical needs, although reversed-phase HPLC is most frequently used because of its wide applicability[32]. Typical chromatographic choices are enumerated in**Table 1**.

Table 1. Selection of chromatographic techniques.

Technique	Typical application
Reversed-phase HPLC	Most drug substances
Normal-phase HPLC	Low–medium polarity, isomers
Ion-exchange chromatography	Ionic compounds
Size-exclusion chromatography	High molecular weight analytes

To provide sufficient retention and sensitivity, suitable columns and detectors are selected after technique selection. While detector selection is based on analyte absorbance or sensitivity requirements, column dimensions, particle size, and flow rate are optimized to achieve acceptable efficiency. While fluorescence and electrochemical detectors are chosen for trace analysis, UV detection is commonly used[33]. Table 2 lists important system parameters.

Table 2. Typical HPLC system parameters.

Parameter	Recommended range
Column length	~25 cm
Particle size	3–5 µm
Flow rate	1.0–1.5 mL/min
Detector	UV / Fluorescence / RI

In order to provide sufficient retention without requiring an excessive amount of run time, the initial chromatographic settings are tuned to keep capacity factors within an acceptable range. By altering the composition of the mobile phase, pH, or ion-pairing agents, selectivity can be further enhanced. This is followed by system parameter tuning to balance resolution and analysis time. Table 3 lists the main phases of technique development.

Table 3. Stages of HPLC method development

Stage	Objective
Initial conditions	Adequate retention
Selectivity optimization	Peak separation
System optimization	Efficiency and speed
Validation	Method reliability

In order to show consistent performance, method validation is carried out in compliance with ICH principles following optimization. System appropriateness, linearity, precision, accuracy, specificity, robustness, limit of detection (LOD), and limit of quantitation (LOQ) are examples of validation parameters. Prior to routine analysis, system suitability checks guarantee adequate chromatographic performance. Stability-indicating techniques are crucial for pharmaceutical applications in order to precisely measure the active ingredient in the presence of contaminants and degradation products produced under stress. These proven HPLC techniques aid in determining shelf life, adhering to regulations, and guaranteeing patient safety and product quality [34,35.] Table 5 summarizes the acceptance criteria and validation parameters.

b. **System Suitability**

Six duplicate injections of the standard solution, which contained 40 µg/mL of Sulbactam and 20 µg/mL of Durlobactam, were used to assess the system's applicability. Retention time, tailing factor, resolution, theoretical plates, and peak area %RSD were among the parameters evaluated. The system performed satisfactorily, as evidenced by the peak areas' %RSD being within 2%.[36].

c. **Specificity**

Blank and placebo solutions were injected to show specificity. The specificity of the approach was confirmed by the absence of interference peaks during the retention times of Durlobactam and Sulbactam[33].

d. **Precision**

Standard working solutions were prepared at the test concentration and analyzed under repeatable conditions in order to assess precision. The percentage RSD of peak regions showed strong technique precision and was within acceptable bounds.

e. **Linearity**

Over the concentration range of 25–150% of the test concentration, linearity was evaluated [37]. Six concentration levels were used to create calibration curves for each analytes, as Table 4 illustrates.

Table 4. Linearity concentration levels.

Level (%)	Sulbactam (µg/mL)	Durlobactam (µg/mL)
25	10	5
50	20	10
75	30	15
100	40	20
125	50	25
150	60	30

f. **Accuracy**

Recovery trials at 50%, 100%, and 150% levels were used to assess accuracy. The pre-analyzed sample solution was spiked with known doses of standard medication. Both medications' recovery percentages fell between 98.0 and 102.0%, demonstrating the method's good accuracy.

g. **Robustness**

Chromatographic conditions, such as flow rate (0.9 and 1.1 mL/min), mobile phase composition, and column temperature (25°C and 35°C), were purposefully varied in order to evaluate robustness. System suitability parameters showed no discernible changes, and %RSD values stayed within permissible bounds [38].

h. Limit of Detection and Limit of Quantitation

Diluted standard solutions of Sulbactam and Durlobactam were prepared in order to calculate LOD and LOQ. The method's sensitivity was confirmed by establishing the concentrations that corresponded to measurable and quantifiable signal levels.

Table 5. Validation parameters and acceptance criteria

Characteristics	**Acceptance Criteria**
Accuracy/trueness	Recovery 98-102% (individual)
Precision	RSD < 2%
Repeatability	RSD < 2%
Intermediate Precision	RSD < 2%
Specificity / Selectivity	No interference
Detection Limit	S/N > 2 or 3
Quantitation Limit	S/N > 10
Linearity	Correlation coefficient R^2 > 0.999
Range	80 –120 %

5.3 Degradation studies:

a. Oxidation:

One milliliter of 20% hydrogen peroxide (H_2O_2) was added independently to one milliliter of the stock solution of sulbactam and durlobactam. The solutions were maintained at 600°C for 30 minutes. The obtained solution was diluted to obtain 40µg/ml and 20µg/ml solutions for the HPLC investigation. Ten microliters were injected into the system, and the chromatograms were recorded to evaluate the sample's stability.

b. Acid Degradation Studies:

1ml of 2N hydrochloric acid was added to 1ml of the stock solution of sulbactam and durlobactam, and the mixture was refluxed for thirty minutes at 600 degrees Celsius. The final solution was diluted to produce 40µg/ml and 20µg/ml solutions. Ten microliters of each solution were injected into the system, and chromatograms were recorded to evaluate the sample's stability.

c. Alkali Degradation Studies:

1ml of 2N sodium hydroxide was added to 1 mL of stock solution Sulbactam and Durlobactam, and the mixture was refluxed at 600 degrees Celsius for thirty minutes. After diluting the resulting solution to obtain 40µg/ml and 20µg/ml solutions, 10 µl were injected into the system, and the chromatograms were recorded to evaluate the sample's stability.

d. Dry Heat Degradation Studies:

To investigate dry heat degradation, the standard medication solution was baked for one hour at 105°C. The resulting solution was diluted to 40µg/ml and 20µg/ml solutions, and 10µl were injected into the system for the HPLC research. The chromatograms were then recorded to evaluate the sample's stability.

e. Photo Stability studies:

The drug's photochemical stability was further investigated by subjecting the 2000µg/ml Sulbactam and 1000µg/ml Durlobactam solution to UV light for one day or 200 Watt hours/m2 in a photostability chamber. The final solution was diluted to produce 40µg/ml and 20µg/ml solutions for the HPLC investigation. Ten microliters were then injected into the system, and the chromatograms were recorded to evaluate the sample's stability.

f. Neutral Degradation Studies:

The medication was refluxed in water at 60º for one hour in order to study stress testing in neutral conditions. The resulting solution was diluted to 40µg/ml and 20µg/ml for the HPLC analysis, and 10 µl were injected into the system. The chromatograms were then recorded to evaluate the sample's stability.

6. RESULTS:

Optimized wavelength selected was 220nm.

6.1 Method development: Method development was done by changing various, mobile phase ratios, buffers etc.

Table 6. Comparison of Chromatographic Conditions Evaluated During HPLC Method Development and Optimization for Simultaneous Estimation of Sulbactam and Durlobactam.

Parameter	Trial 1	Trial 2	Trial 3	Trial 4	Optimized Method
Detection Wavelength	220 nm	220 nm	220 nm	220 nm	**220 nm**
Mobile Phase	Methanol : Water (50:50	Acetonitrile : Water (50:50 v/v)	Acetonitrile : 0.01 N	Acetonitrile : 0.1% OPA	**0.1% Orthophosphoric**

	v/v)		KH_2PO_4 (50:50 v/v)	(50:50 v/v)	**Acid : Acetonitrile (70:30 v/v)**
Flow Rate	1.0 mL/min	1.0 mL/min	1.0 mL/min	1.0 mL/min	**1.0 mL/min**
Column	Discovery C18 (4.6 × 250 mm, 5 µm)	Discovery C18 (4.6 × 250 mm, 5 µm)	Discovery C18 (4.6 × 250 mm, 5 µm)	Discovery C18 (4.6 × 250 mm, 5 µm)	**Agilent C18 (4.6 × 250 mm, 5 µm)**
Column Temperature	30 °C	30 °C	30 °C	30 °C	**30 °C**
Injection Volume	10 µL	10 µL	10 µL	10 µL	**10 µL**
Run Time	10 min	10 min	10 min	10 min	**5 min**
Diluent	Water : Acetonitrile (50:50)	Water : Acetonitrile (50:50)	Water : Acetonitrile (50:50)	Water : Acetonitrile (50:50)	**Water : Acetonitrile (50:50)**
Observations / Results	Only Durlobactam eluted; peak did not meet system suitability criteria	Both peaks eluted; long retention time with broad peak shapes	Retention times higher than reported in literature	Good peak shapes but retention time higher than literature	**Both peaks showed good resolution, acceptable tailing factor, theoretical plate count, and resolution**
Conclusion	Method not suitable	Method not suitable	Method not suitable	Method not suitable	**Method optimized and finalized**

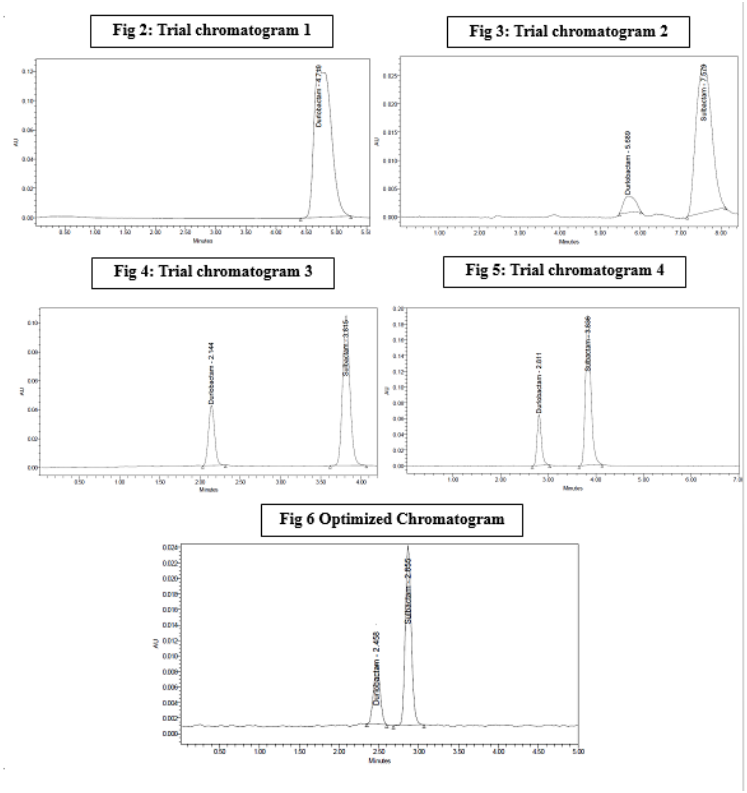


Figure 7: HPLC method development and optimization chromatograms for Sulbactam and Durlobactam.

Observation:

Trial chromatograms (Fig. 1–Fig. 4) illustrate the progressive optimization of chromatographic conditions, showing variations in retention time, peak shape, and resolution for Sulbactam and Durlobactam under different experimental conditions. The optimized chromatogram demonstrates well-resolved, symmetric peaks with acceptable retention times for Durlobactam (~2.45 min) and Sulbactam (~2.85 min), indicating the suitability of the finalized HPLC conditions for simultaneous estimation of both analytes.

6.2 System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines

Table 7: System suitability parameters for Sulbactam and Durlobactam.

S no	**Durlobactam**			**Sulbactam**			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resoluton
1	2.459	4525	1.13	2.551	7139	0.66	2.8
2	2.461	4640	1.09	2.851	7252	1.02	2.8
3	2.462	4559	1.31	2.853	7249	1.12	2.9
4	2.464	4469	1.07	2.853	7270	1.06	2.8
5	2.466	4513	0.79	2.854	7210	1.16	2.9
6	2.573	4638	1.0	2.854	7339	1.29	2.8

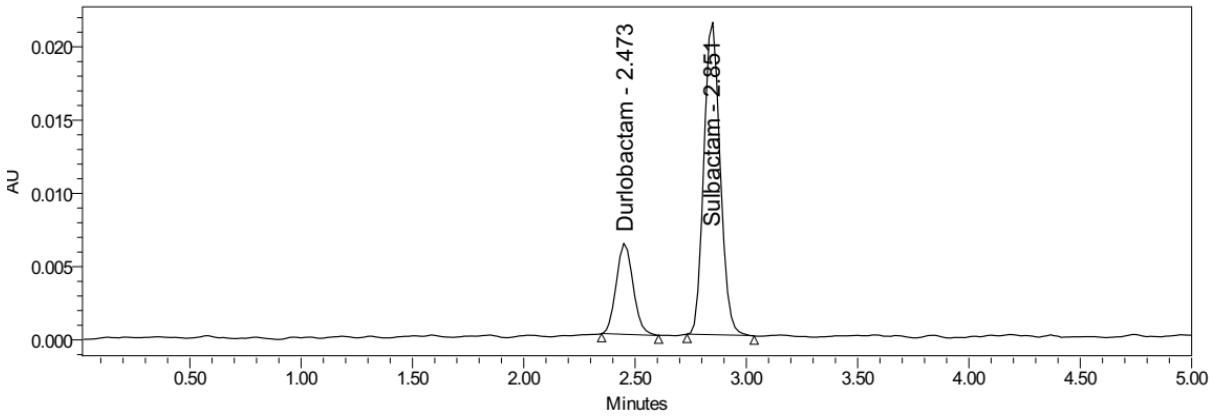


Fig 8: System suitability Chromatogram

Discussion: According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

6.3 Specificity:

Fig 9: Chromatogram of blank

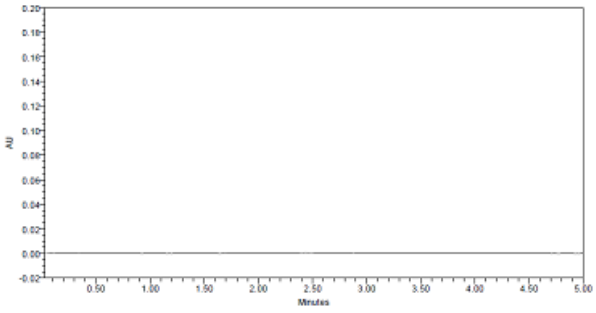
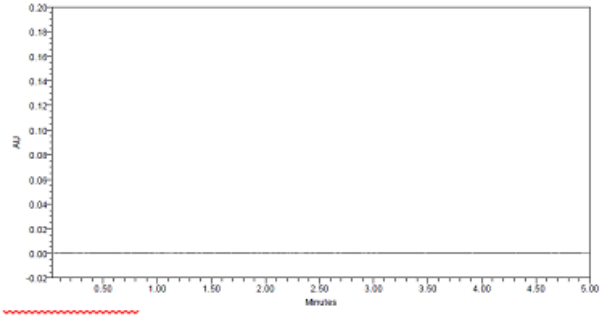


Fig 10: Chromatogram of



Discussion: Retention times of Sulbactam and Durlobactam were 2.185 min and 2.853 min respectively. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

6.4 Linearity:

Table 8: Linearity table for Sulbactam and Durlobactam.

Sulbactam		Durlobactam	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
10	26751	5	10837
20	54650	10	21763
30	81722	15	32560
40	108495	20	43382
50	135233	25	54340
60	160283	30	64287

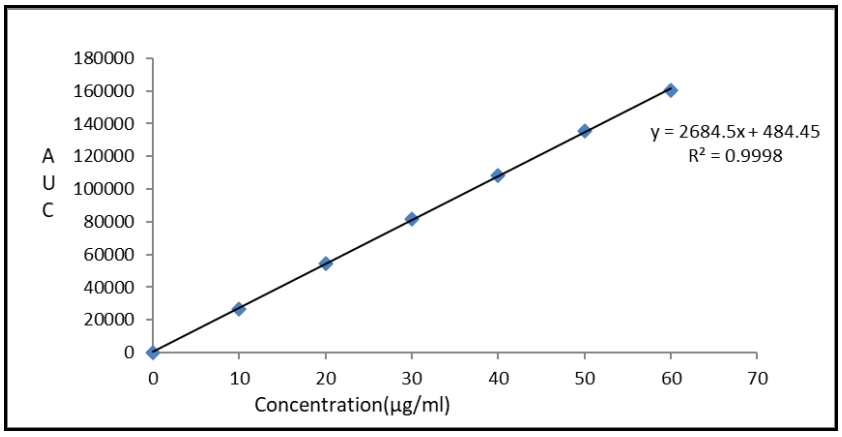


Fig 11: Calibration curve of Sulbactam.

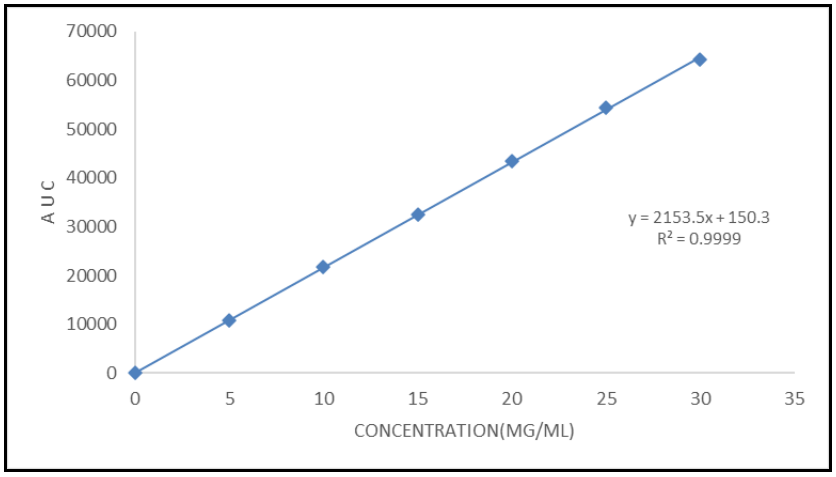


Fig 12: Calibration curve of Durlobactam

Discussion: Six linear concentrations of Sulbactam (10- 60µg/ml) and Durlobactam (5-30µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Sulbactam was y = 2684.5x + 484.45.and of Durlobactam was y = 2153.5 + 150.3 Correlation coefficient obtained was 0.999 for the both drugs.

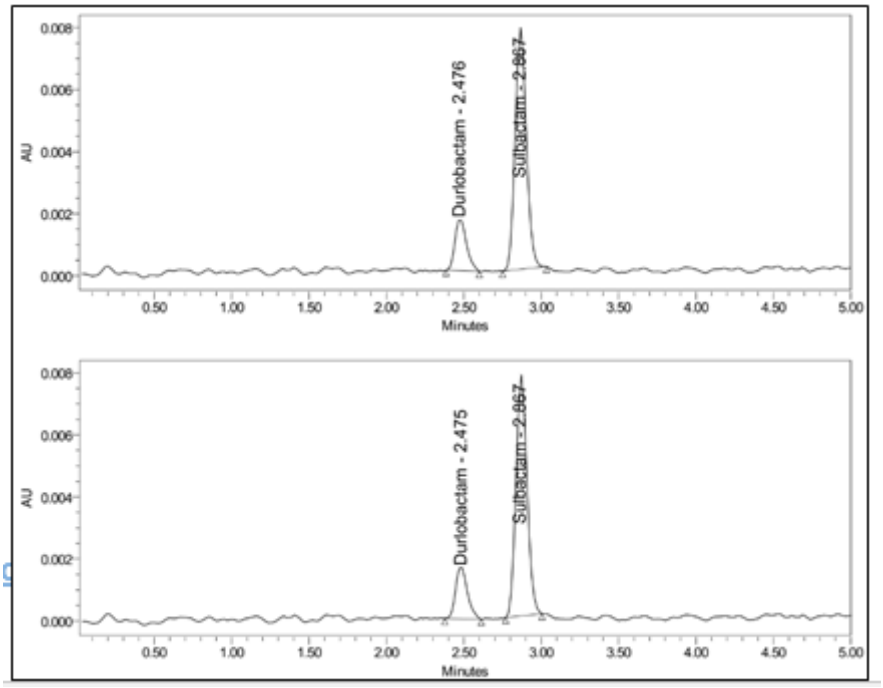


Fig 13: Linearity 25% Chromatogram of Sulbactam and Durlobactam

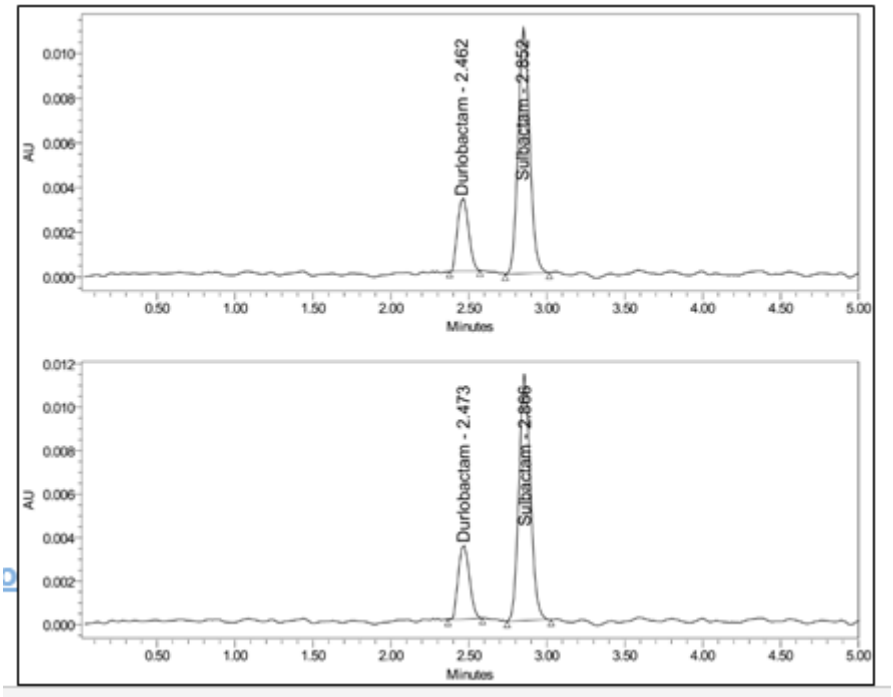


Fig 14: Linearity 50% Chromatogram of Sulbactam and Durlobactam.

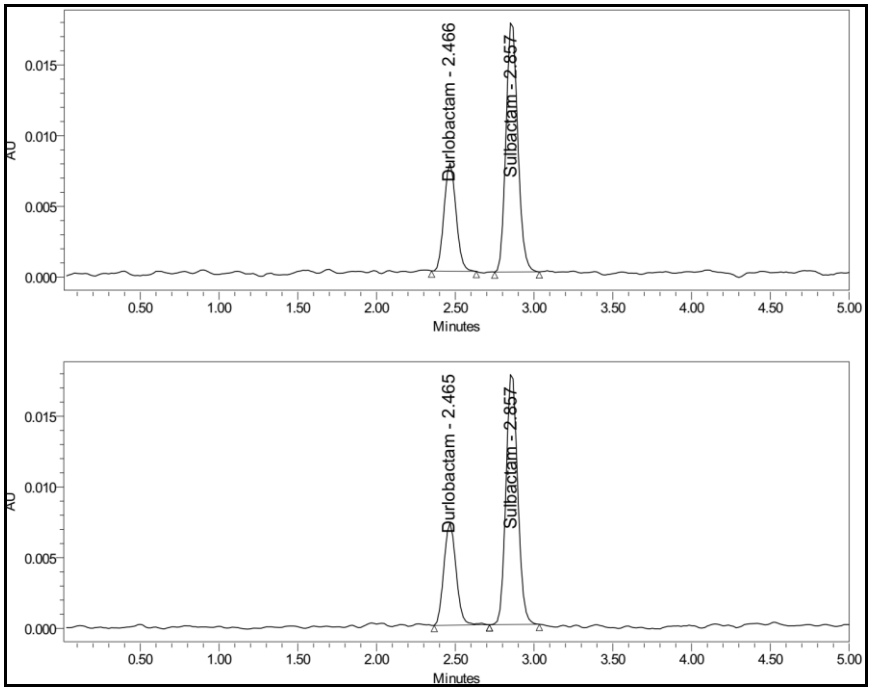


Fig 15: Linearity 75% Chromatogram of Sulbactam and Durlobactam.

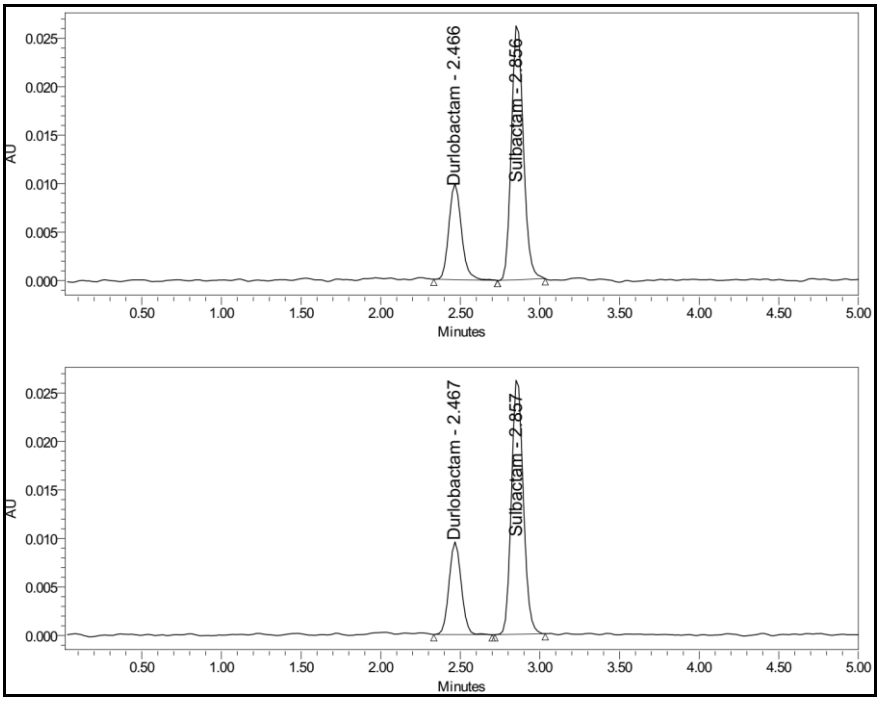


Fig 16: Linearity 100% Chromatogram of Sulbactam and Durlobactam.

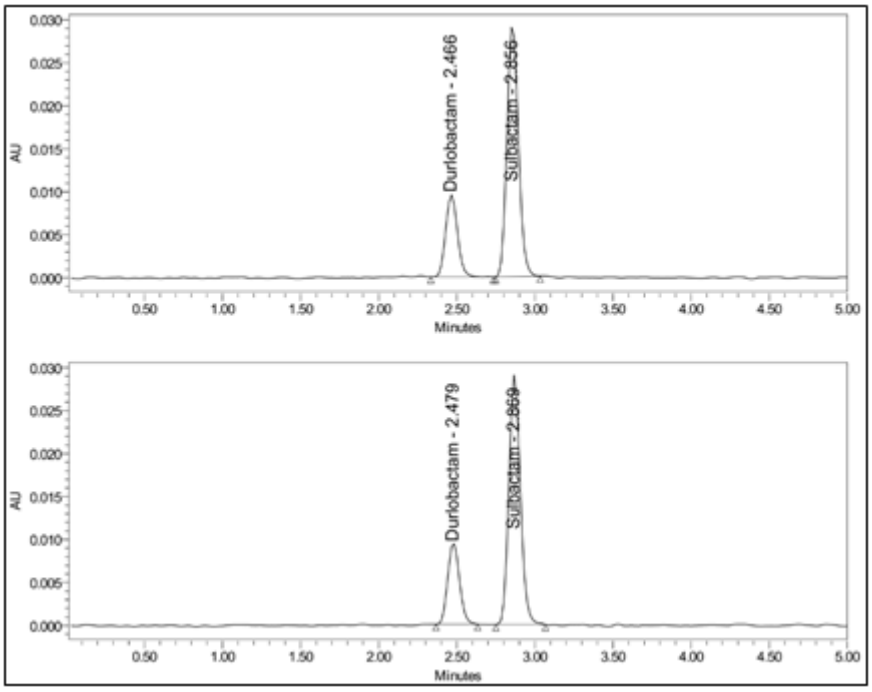


Fig 17 Linearity 125% Chromatogram of Sulbactam and Durlobactam.

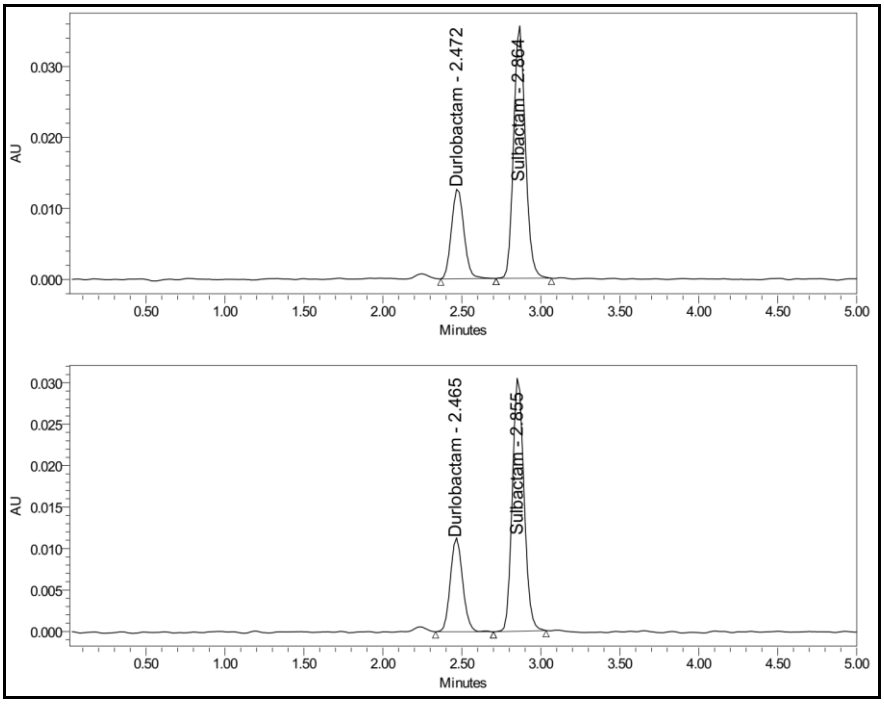


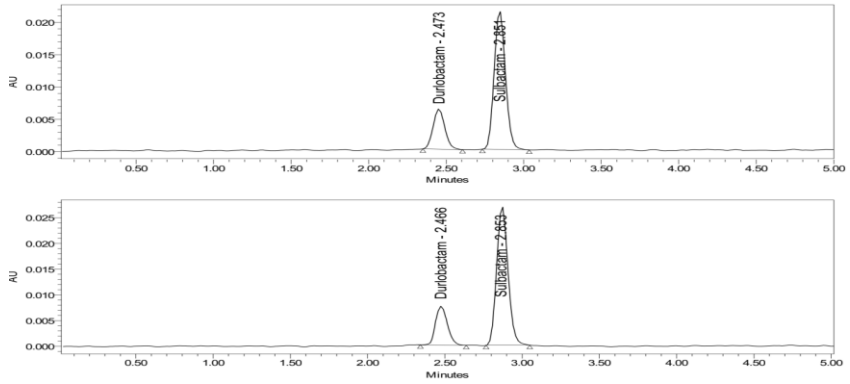
Fig 18 Linearity 150% Chromatogram of Sulbactam and Durlobactam.

6.5 Precision:

a. System Precision:

Table 9: System precision table of Sulbactam and Durlobactam

S. No	Area of Sulbactam	Area of Durlobactam
1	357170	136302
2	359298	133599
3	350388	134750
4	357945	135087
5	355128	133755
6	355640	136196
Mean	355928	134948
S.D	3110.7	1156.9
%RSD	0.9	0.9



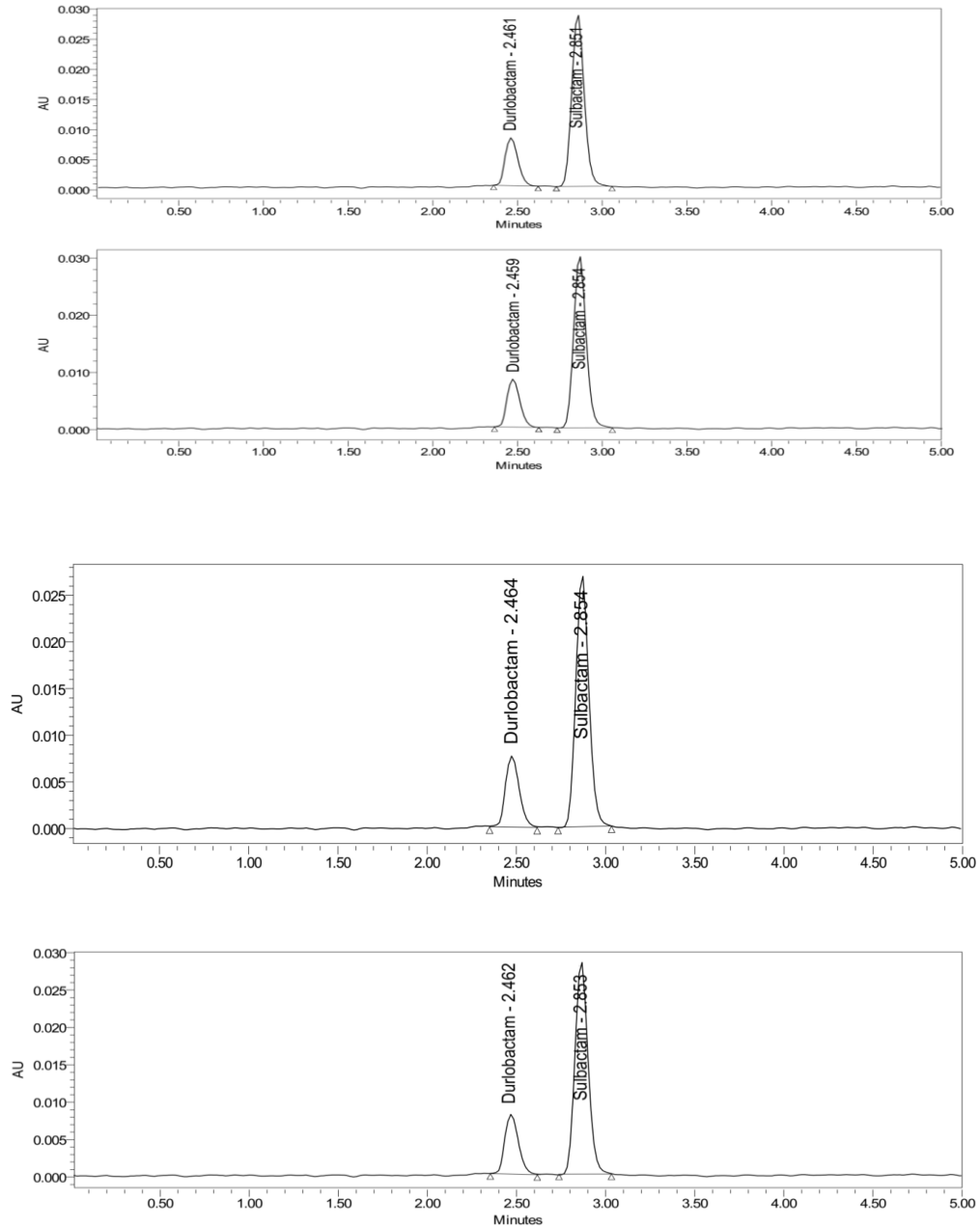


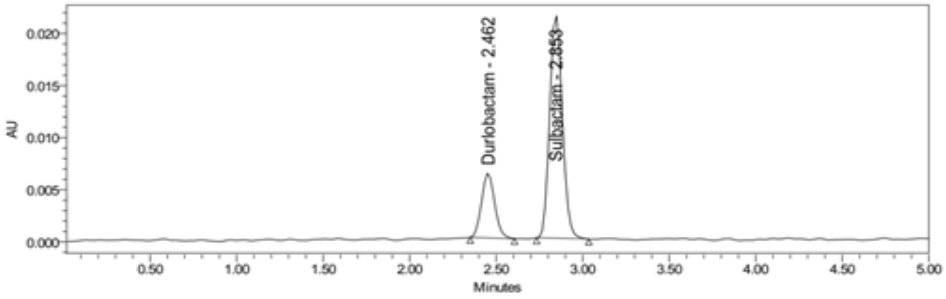
Fig 19 System precision chromatogram

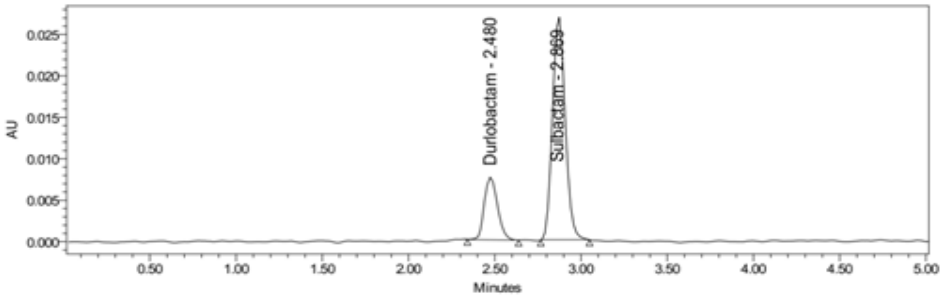
Discussion: Six injections of the working standard solution were made from a single volumetric flask, and the areas that were obtained were described above. For two medications, average area, standard deviation, and percentage RSD were computed. The percentage RSD for sulbactam and durlobactam was found to be 0.3% and 0.6%, respectively. Because the precision limit was less than "2," this approach passed the system precision.

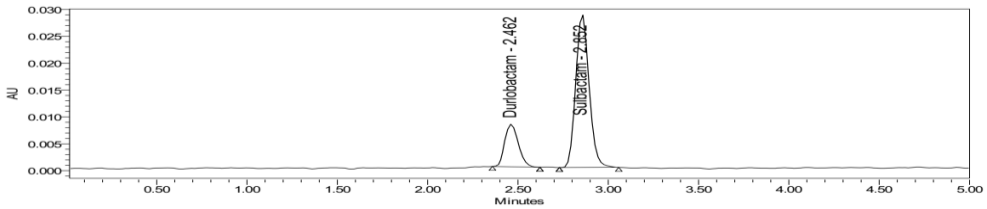
6.6 Repeatability:

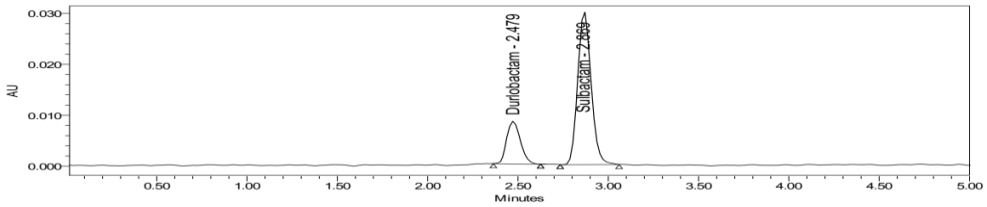
Table 10: Repeatability table of Sulbactam and Durlobactam.

S. No	Area of Sulbactam	Area of Durlobactam
1	108386	43294
2	108275	43170
3	107906	43305
4	108642	43171
5	108352	43327
6	108737	43660
Mean	108383	43321
S.D	294.1	179.6
%RSD	0.3	0.4









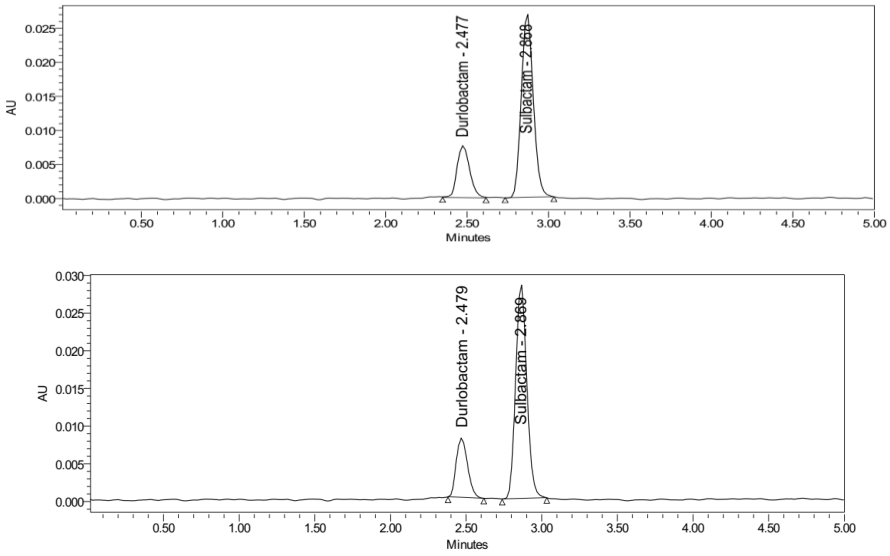


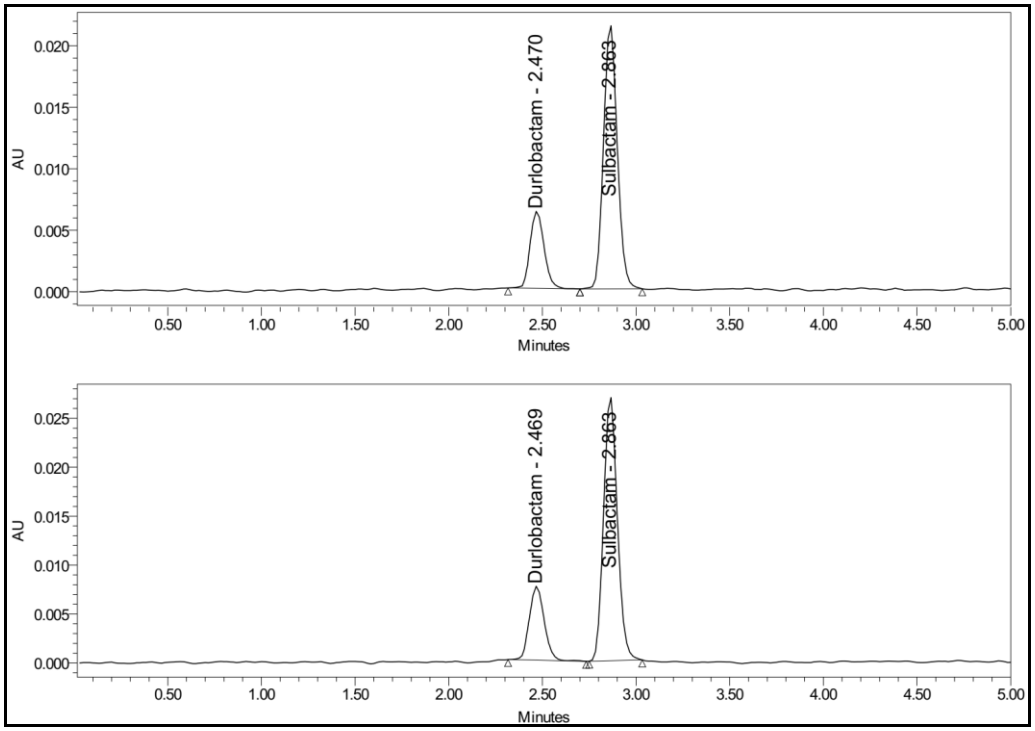
Fig 20: Repeatability chromatogram.

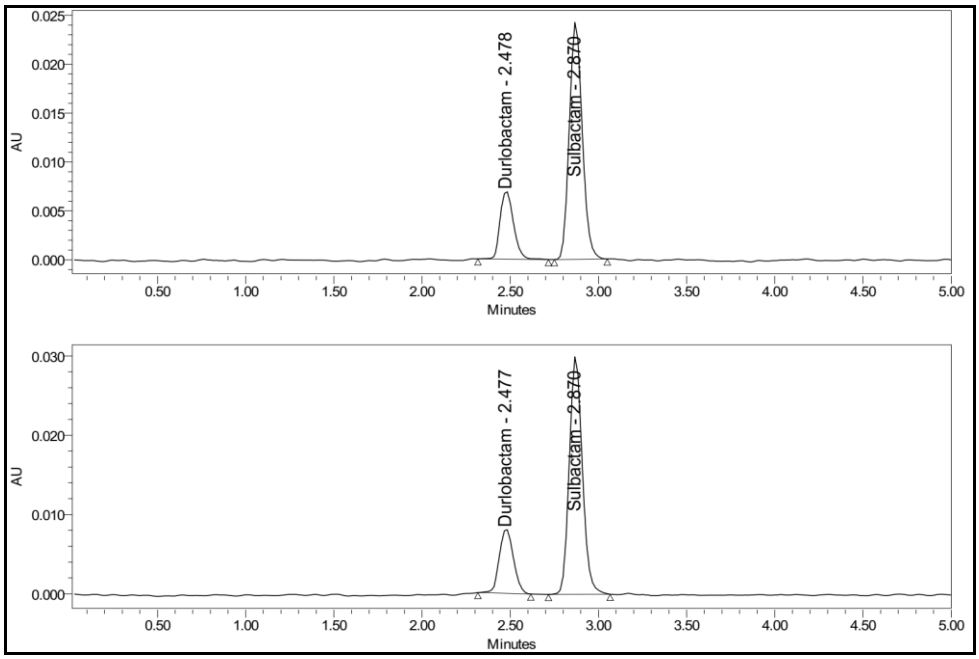
Discussion: Six working sample solutions of the same concentrations were created by multiple sampling from a sample stock solution; each injection from each working sample solution was administered, and the obtained areas were listed in the above table. Sulbactam and Durlobactam yielded average area, standard deviation, and percentage RSD of 0.3% and 0.5%, respectively. The system precision was passed using this approach since the precision limit was less than "2."

Intermediate precision (Day_ Day Precision):

Table 6.5 Intermediate precision table of Sulbactam and Durlobactam.

S. No	Area of Sulbactam	Area of Durlobactam
1	106321	43406
2	106121	43194
3	105712	43153
4	106520	43378
5	105318	42969
6	106112	43186
Mean	106017	43214
S.D	435.2	160.4
%RSD	0.4	0.4


Durlobactam - 2.470
Sulbactam - 2.863
AU
Minutes
Durlobactam - 2.469
Sulbactam - 2.863
AU
Minutes


Durlobactam - 2.478
Sulbactam - 2.870
AU
Minutes
Durlobactam - 2.477
Sulbactam - 2.870
AU
Minutes

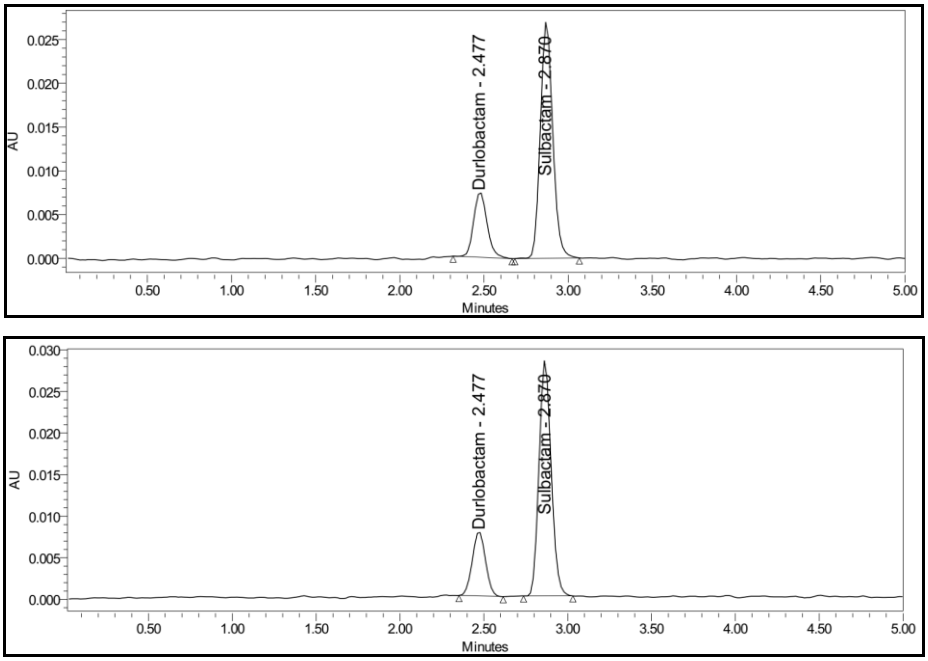


Fig 21: Inter Day precision Chromatogram

Discussion: Six working sample solutions of the same concentrations were prepared by multiple sampling from a sample stock solution; each injection from each working sample solution was administered the following day of sample preparation, and the acquired areas are listed in the above table. Sulbactam and Durlobactam had average area, standard deviation, and percentage RSD of 0.4% and 0.4%, respectively. In this manner, the system precision was passed because the precision limit was less than "2."

6.7 Accuracy:

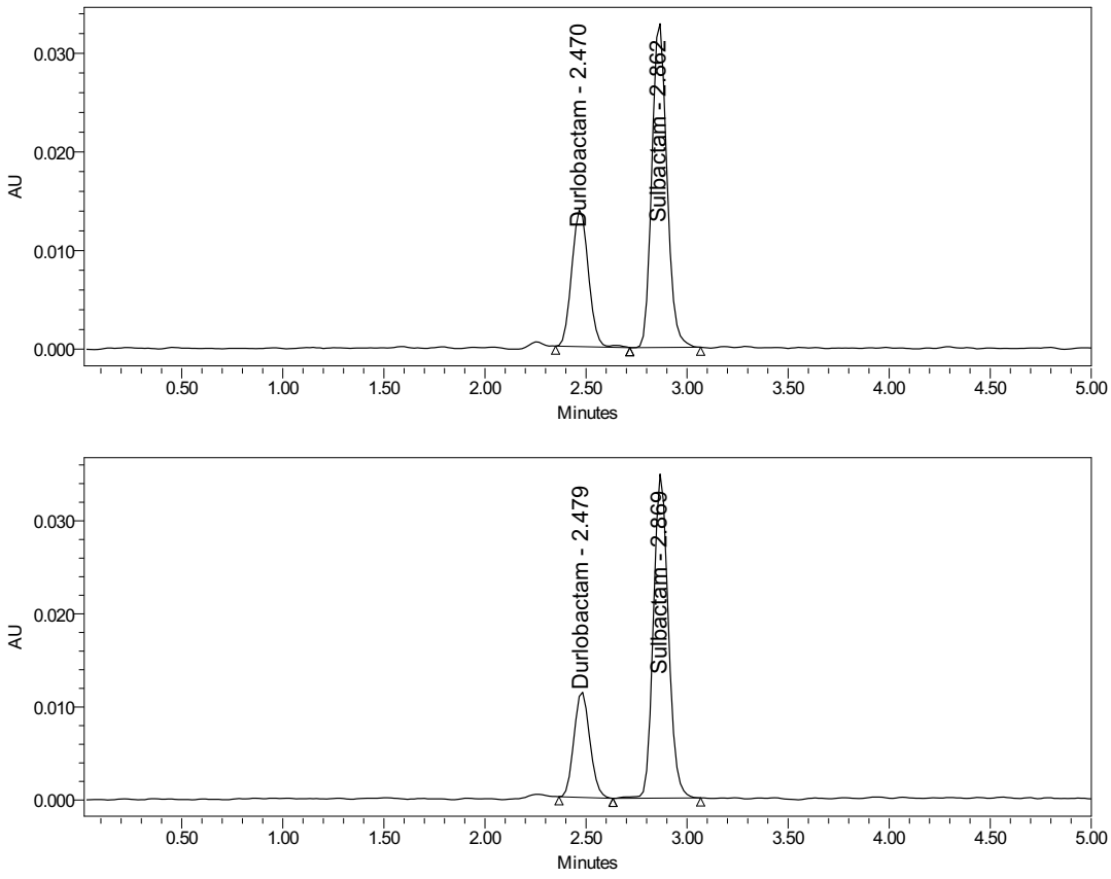
Table 6.6 Accuracy table of Sulbactam.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	20	19.79	98.94	99.29%
	20	19.76	98.81	
	20	20.13	100.66	
100%	40	39.57	98.93	
	40	39.85	99.63	
	40	39.69	99.23	
150%	60	59.54	99.23	
	60	59.36	98.93	
	60	59.53	99.22	

Table 6.7 Accuracy table of Durlobactam.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	10	10.05	100.55	99.87%
	10	9.93	99.3	
	10	9.96	99.56	
100%	20	20.15	100.73	
	20	19.98	99.9	
	20	20.08	100.38	
150%	30	29.54	98.48	
	30	29.75	99.16	
	30	30.23	100.78	

Discussion: The conventional addition procedure was used to prepare three levels of accuracy samples. For each degree of accuracy, three injections were administered, and the mean percentage recovery for sulbactam and durlobactam was 99.29% and 99.87%, respectively.



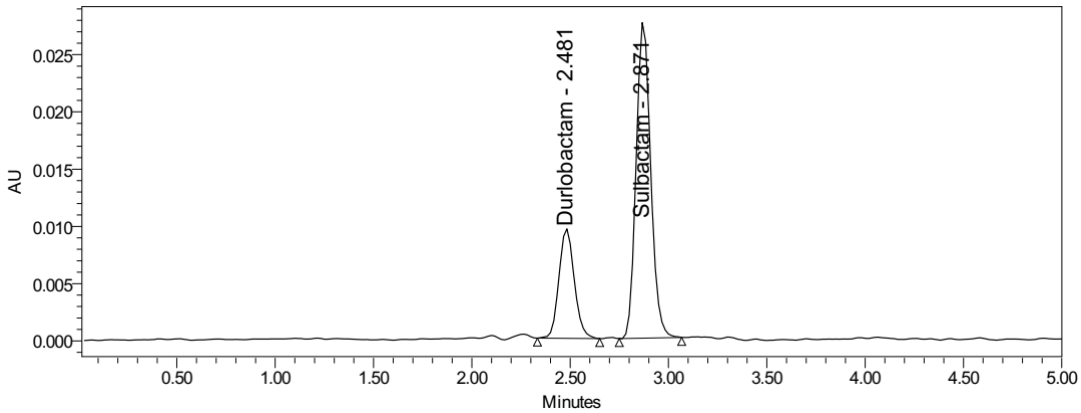


Fig 22: Accuracy 50% Chromatogram of Sulbactam and Durlobactam.

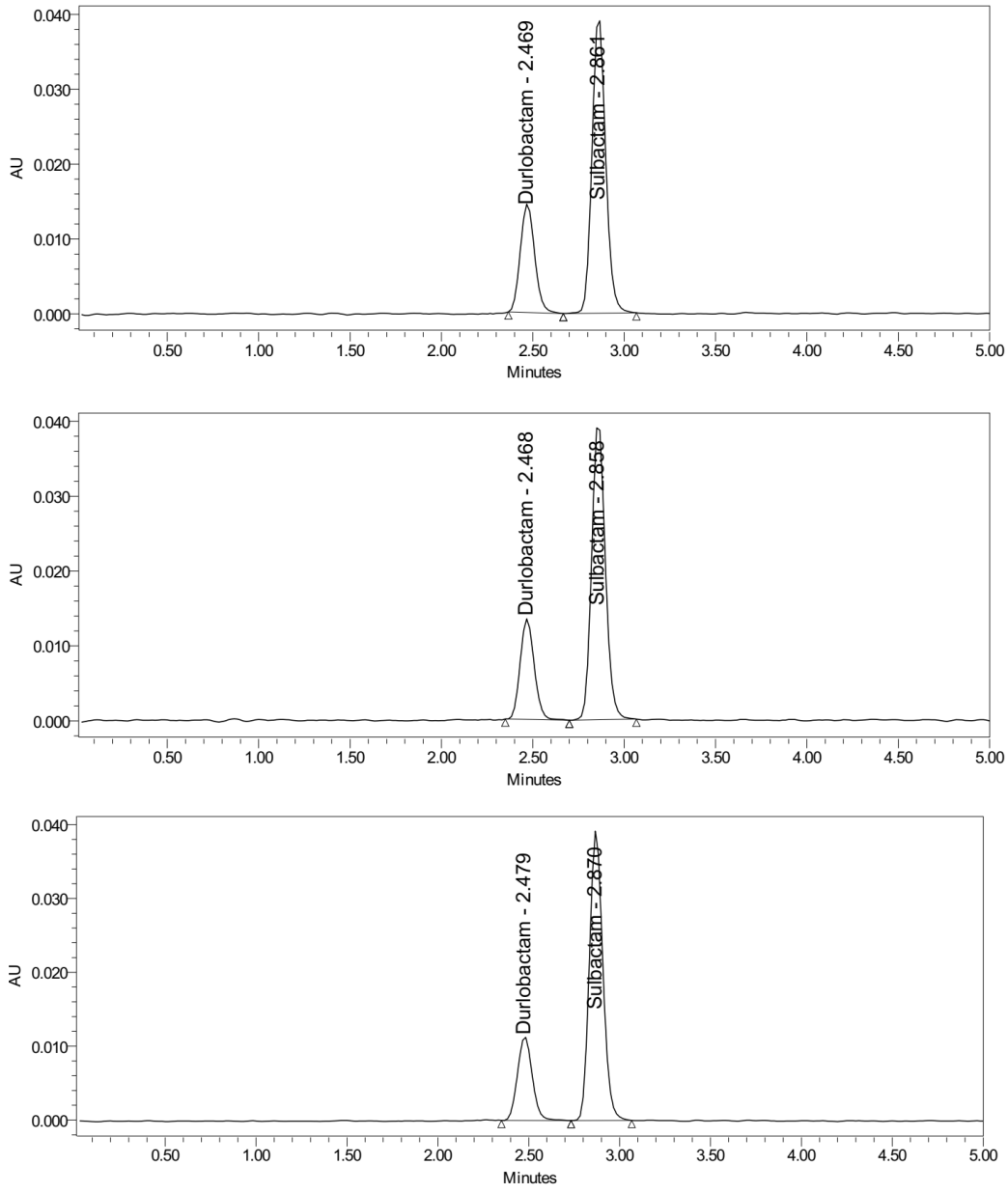


Fig 23: Accuracy 100% Chromatogram of Sulbactam and Durlobactam

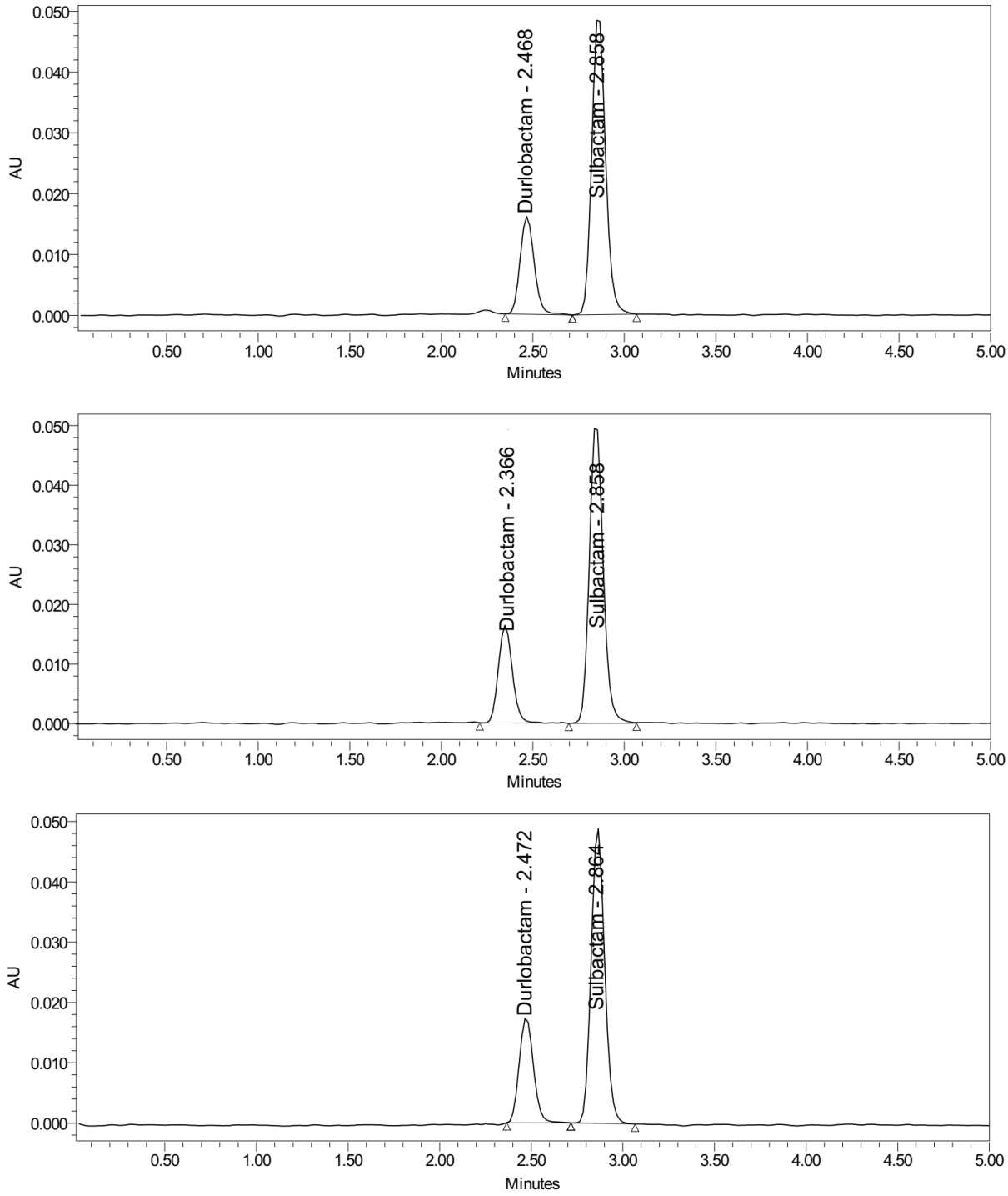


Fig 24: Accuracy 150% Chromatogram of Sulbactam and Durlobactam

6.8 Sensitivity:

Table 6.8 Sensitivity table of Sulbactam and Durlobactam

Molecule	LOD	LOQ
Sulbactam	0.09	0.27
Durlobactam	0.04	0.13

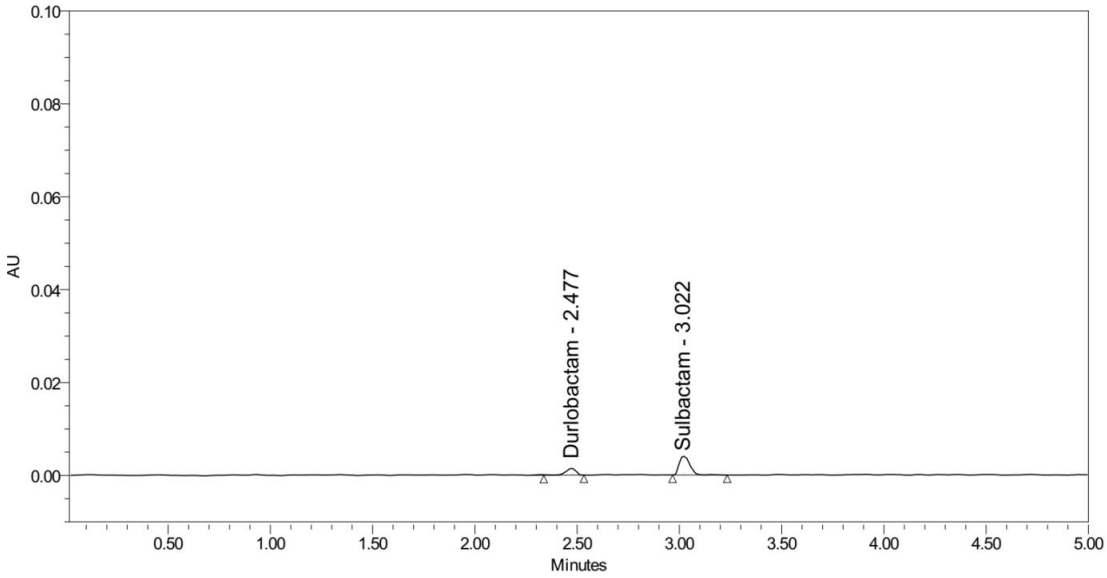


Fig. 25 LOD Chromatogram of Standard.

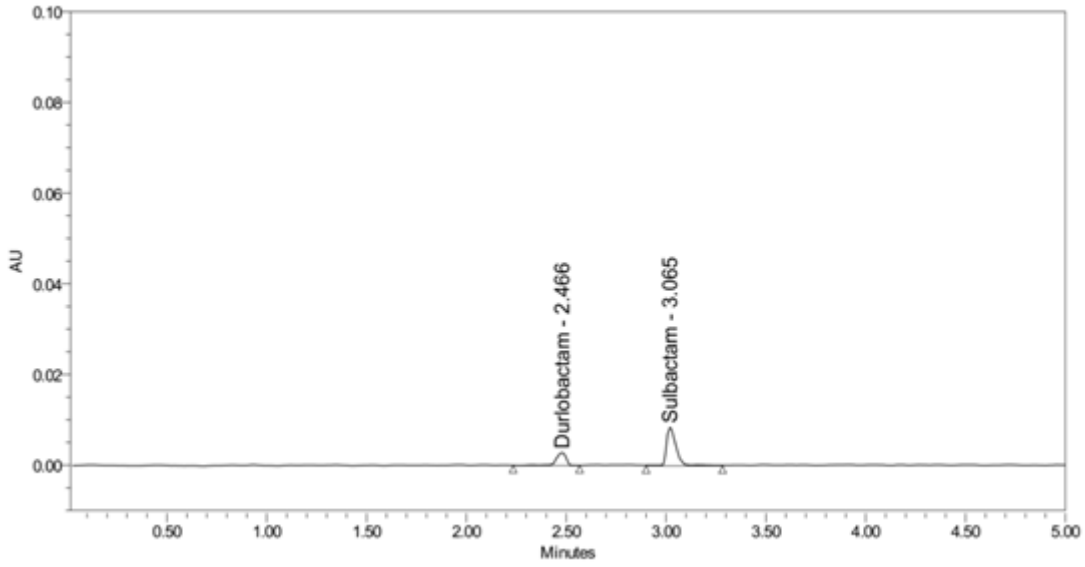


Fig.26: LOQ Chromatogram of Standard

6.9 Robustness:

Table 6.9 Robustness data for Sulbactam and Durlobactam.

S.no	Condition	%RSD of Sulbactam	%RSD of Durlobactam
1	Flow rate (-) 0.9ml/min	0.5	0.5
2	Flow rate (+) 1.1ml/min	0.1	0.2
3	Mobile phase (-) 75B:25A	0.2	0.5
4	Mobile phase (+) 65B:35A	0.2	0.2
5	Temperature (-) 25°C	0.1	0.3
6	Temperature (+) 35°C	0.1	0.5

Discussion: Samples were injected in duplicate while robustness conditions such as Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (75B:25A), mobile phase plus (65B:35A), temperature minus (25°C) and temperature plus (35°C) were maintained. All of the system suitability parameters passed and were not significantly impacted. %RSD was within the permitted range.

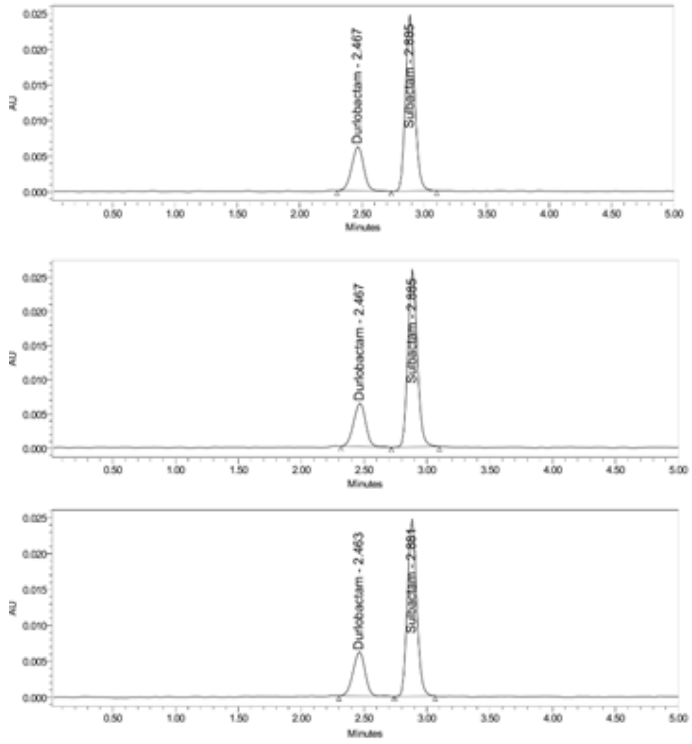
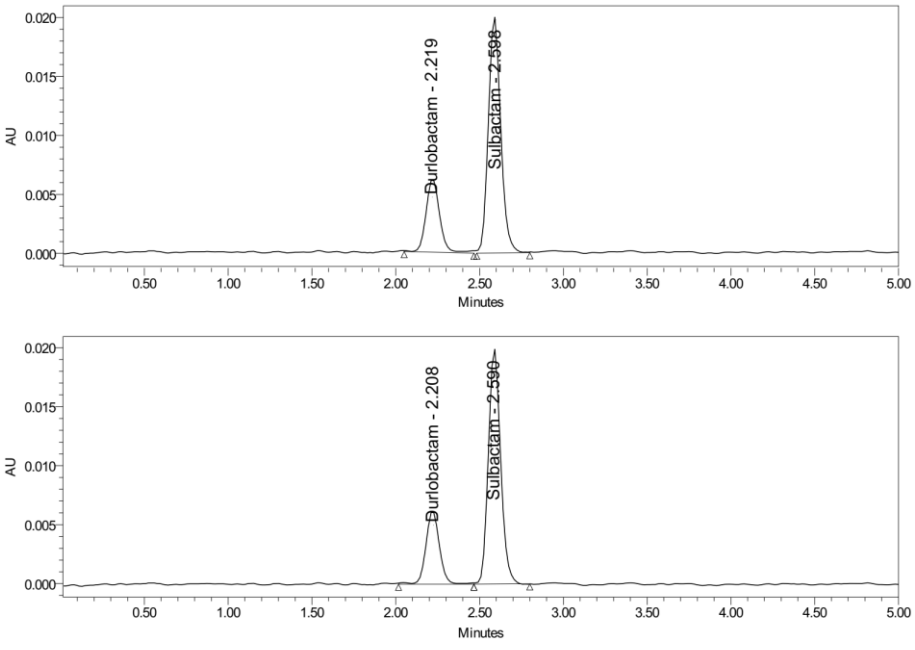


Fig 27 Flow minus Chromatogram of Sulbactam and Durlobactam.



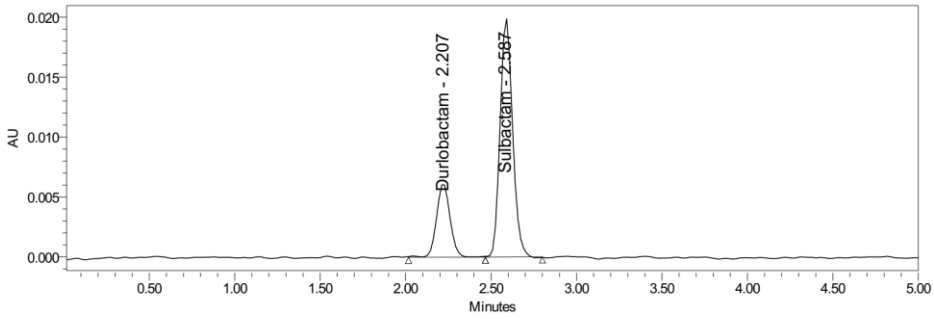


Fig 28: Flow plus Chromatogram of Sulbactam and Durlobactam.

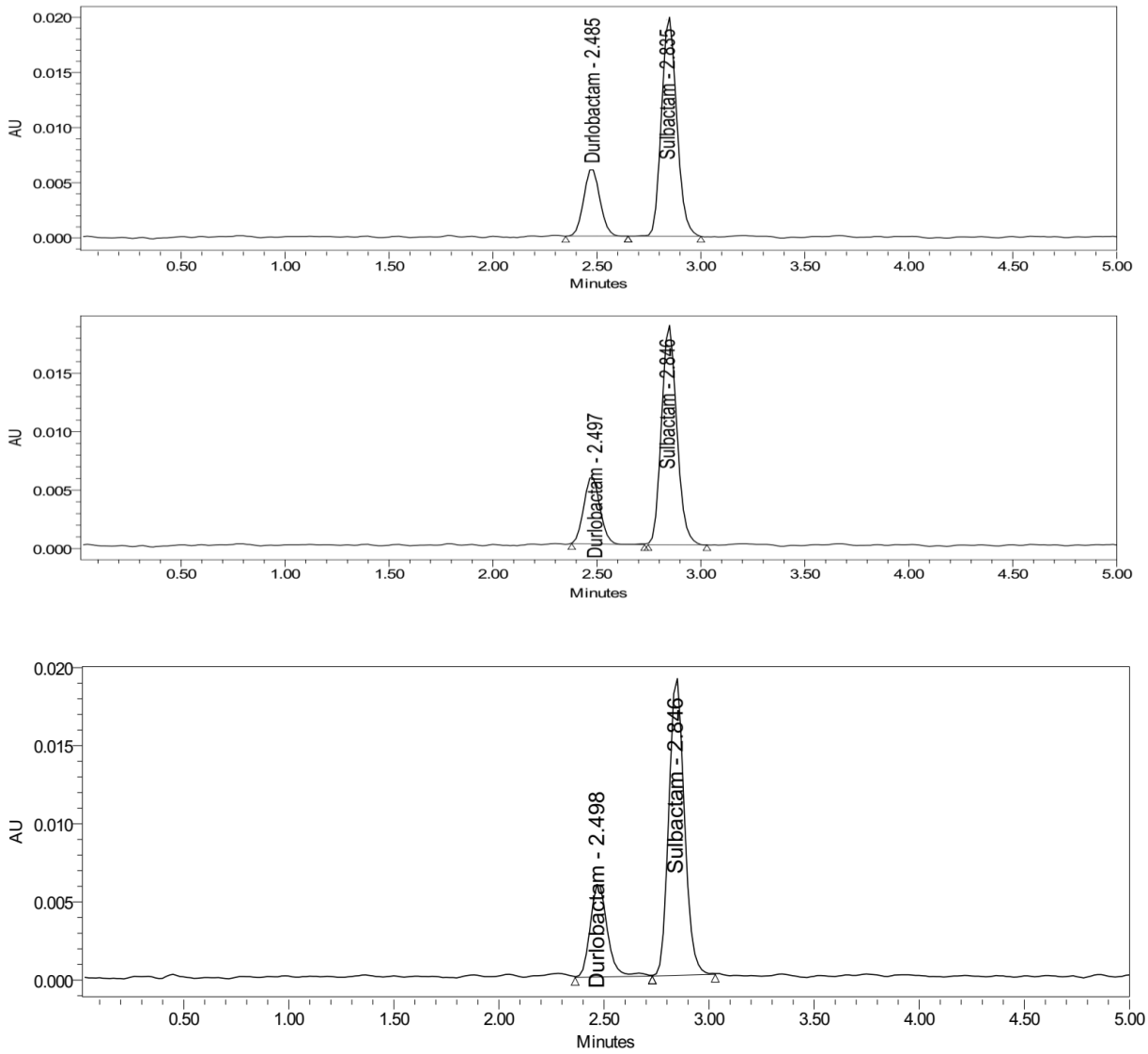


Fig 29: Mobile phase minus Chromatogram of Sulbactam and Durlobactam.

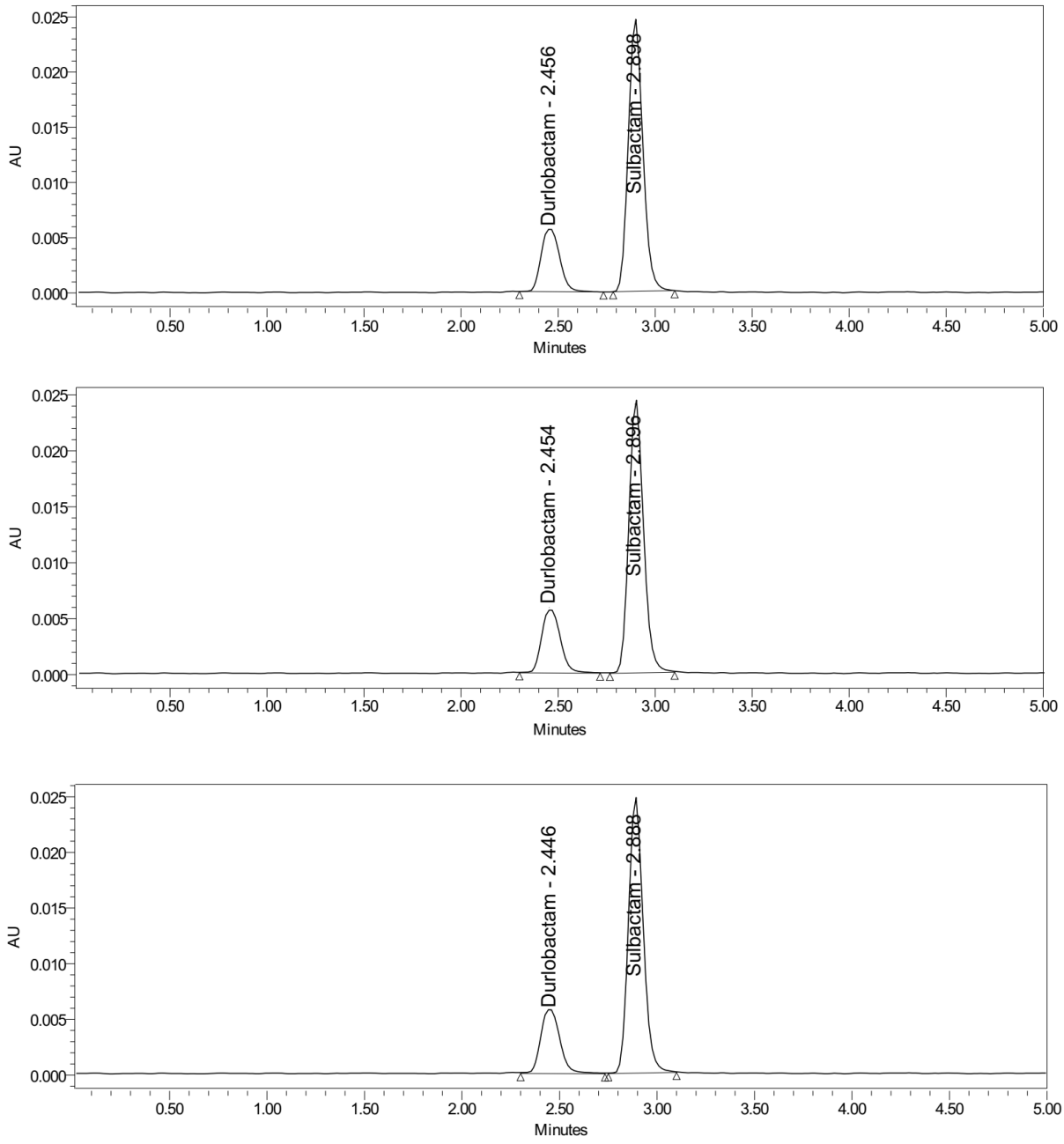


Fig 30: Mobile phase Plus Chromatogram of Sulbactam and Durlobactam.

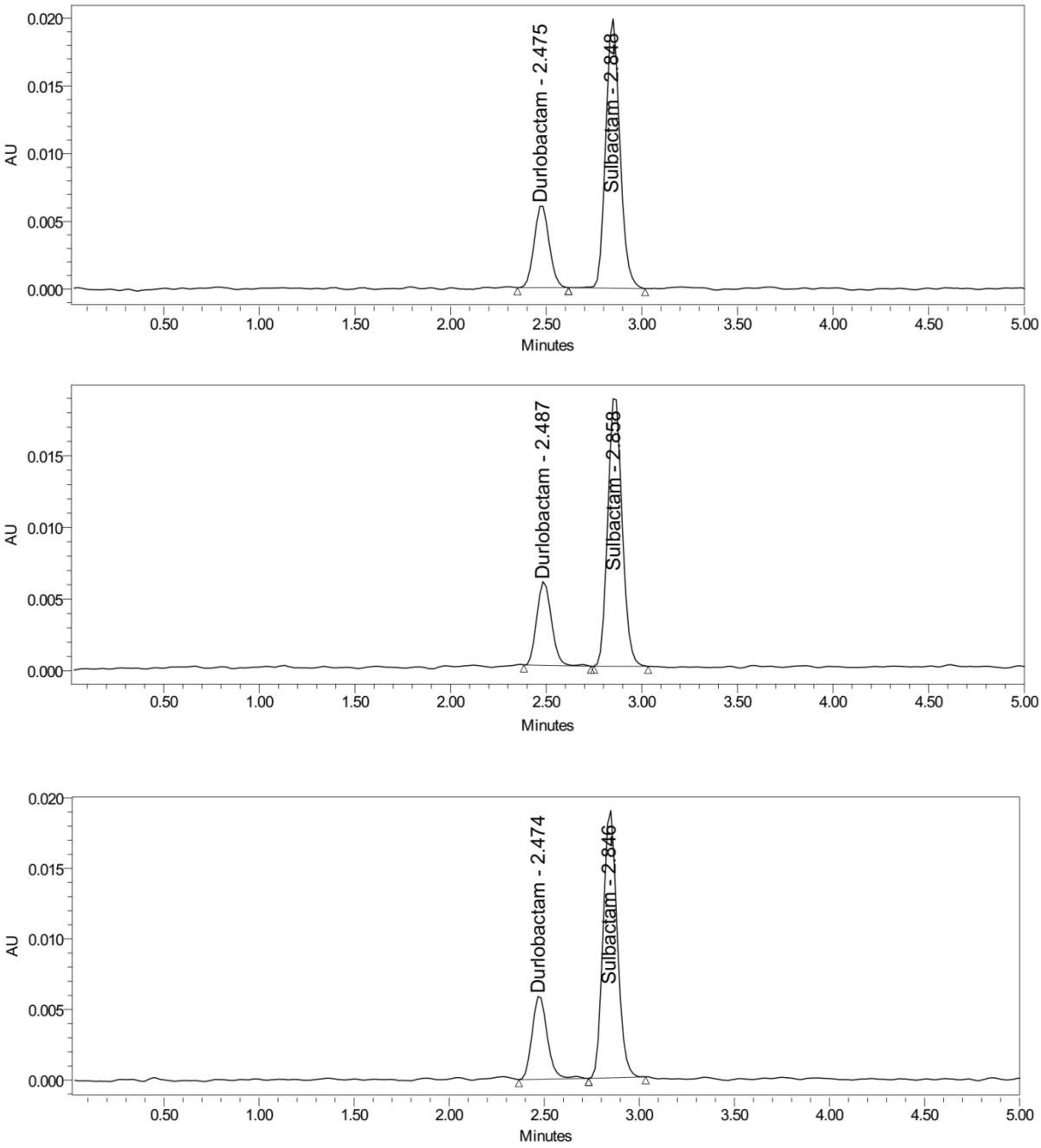


Fig 31: Temperature minus Chromatogram of Sulbactam and Durlobactam

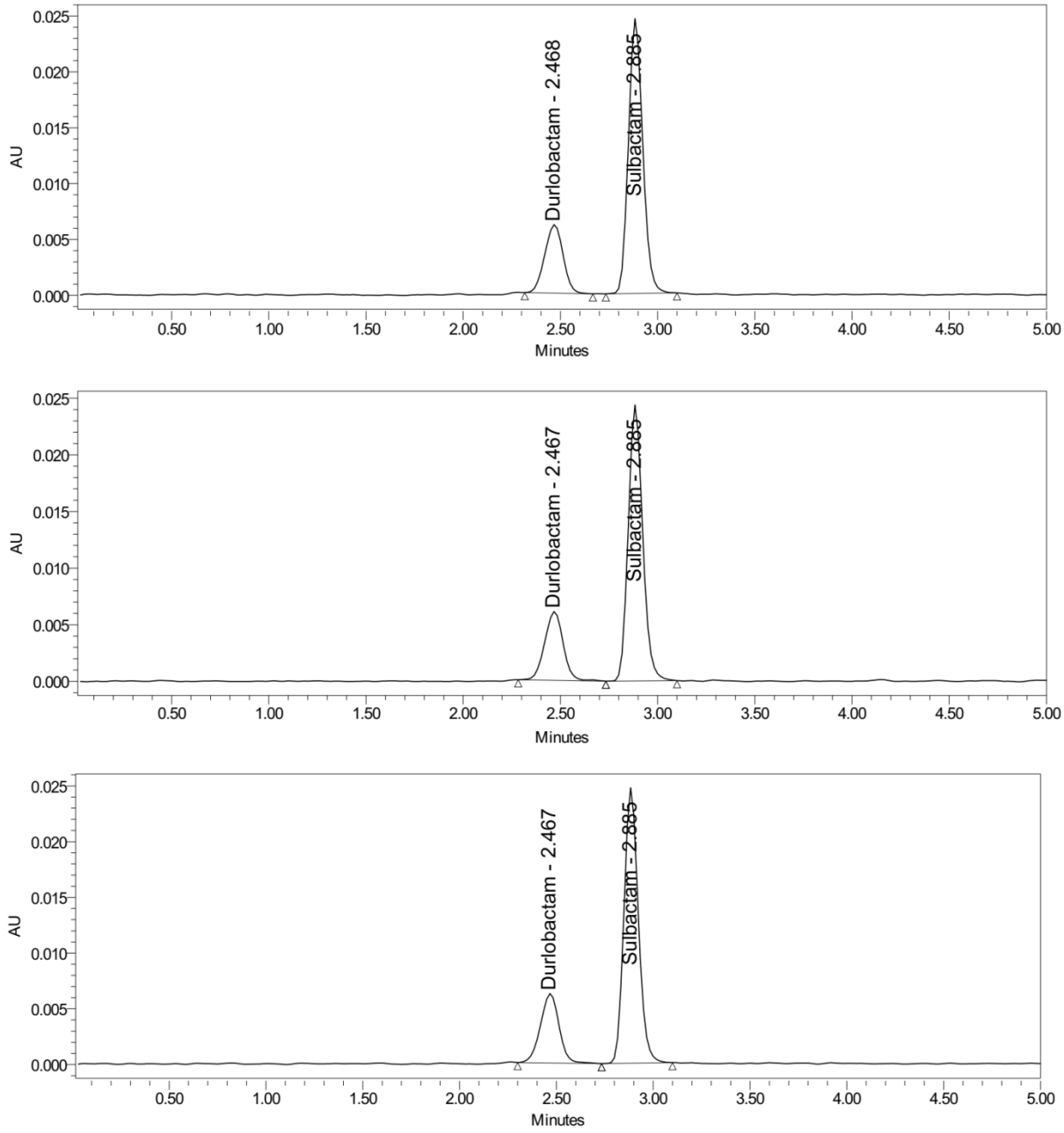


Fig 32: Temperature plus Chromatogram of Sulbactam and Durlobactam

Assay: with the labels "Sulbactam and Durlobactam 1 g, 0.5 g/vail injection." The aforementioned formulation was used for the assay. The average assay results for durlobactam and sulbactam were 99.34% and 99.48%, respectively.

Table 6.10 Assay Data of Sulbactam.

S.no	Standard Area	Sample area	% Assay
1	108970	108386	99.55
2	108187	108275	99.45
3	108418	107906	99.11
4	108714	108642	99.79

5	109151	108352	99.52
6	108502	108737	99.87
Avg	108657	108383	99.48
Stdev	359.8	294.1	0.27
%RSD	0.3	0.3	0.3

Table 6.11 Assay Data of Durlobactam

S.no	Standard Area	Sample area	% Assay
1	43440	43294	99.27
2	43815	43170	98.99
3	43254	43305	99.3
4	43673	43171	98.99
5	43187	43327	99.35
6	43247	43660	100.11
Avg	43436	43321	99.34
Stdev	257.2	179.6	0.412
%RSD	0.6	0.4	0.41

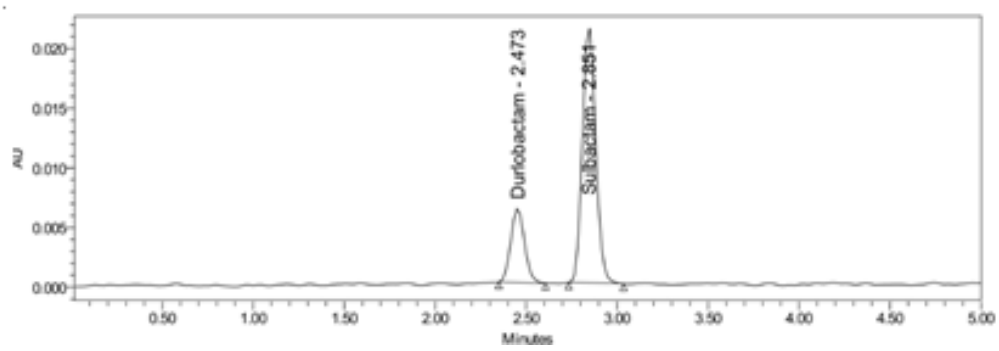


Fig 33: Chromatogram of working standard solution

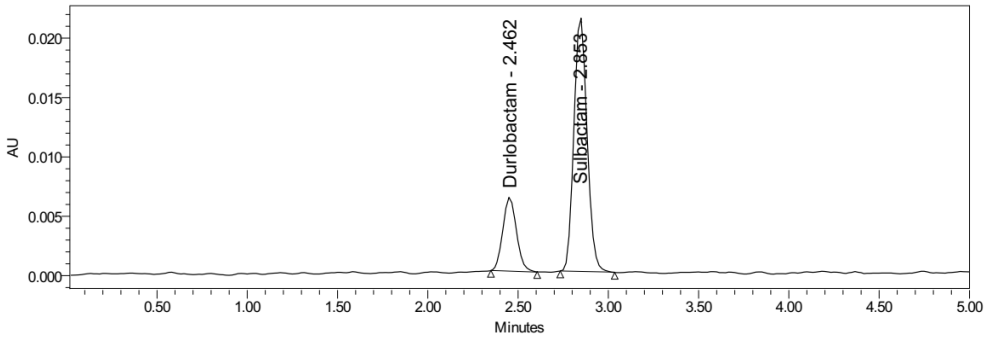


Fig 34: Chromatogram of working sample solution

7. Degradation studies: The proportion of drug degradation in solution is determined by injecting standards and degraded samples under various conditions, including acid, alkali, oxidative, photolytic, thermal, and neutral analysis.

Table 6.12. degradation data

Type of degradation	Sulbactam			Durlobactam		
	Area	%Recovered	% Degraded	Area	%Recovered	% Degraded
Acid	101632	93.35	6.65	41861	95.99	4.01
Base	102889	94.50	5.50	41367	94.86	5.14
Peroxide	100565	92.37	7.63	41172	94.41	5.59
Thermal	105848	97.22	2.78	42572	97.62	2.38
Uv	106548	97.86	2.14	42812	98.17	1.83
Water	108471	99.63	0.37	43269	99.22	0.78

7.1 Degradation chromatograms

Acid degradation chromatogram

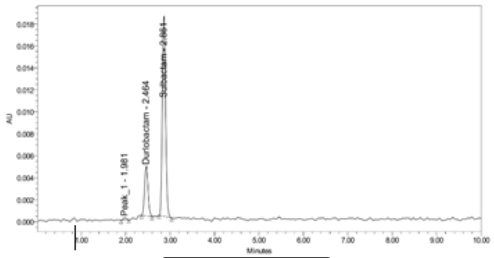


Fig35: acid

Base degradation chromatogram

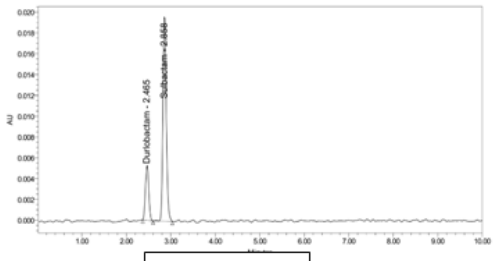


Fig36: base

Peroxide degradation chromatogram

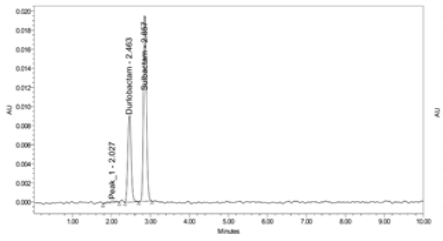


Fig 37: peroxide degradation

Thermal degradation chromatogram

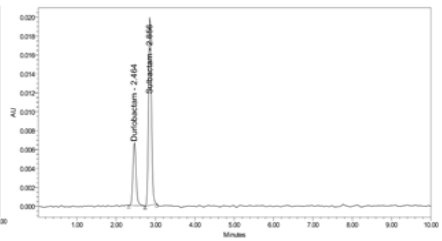


Fig 38: Thermal degradation chromatogram

UV degradation chromatogram

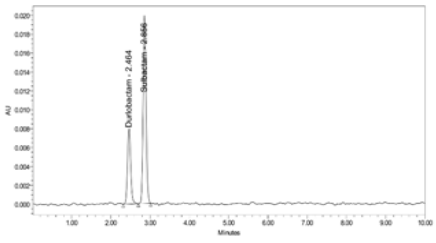


Fig.39: UV degradation chromatogram

Water degradation chromatogram

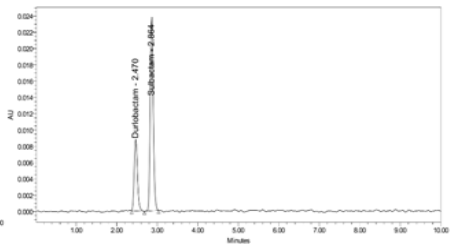


Fig.40: Water degradation chromatogram

8. SUMMARY

Parameters		Sulbactam	Durlobactam	LIMIT
Linearity Range (µg/ml)		10-60 µg/ml	5-30 µg/ml	R< 1
Regression coefficient		0.999	0.999	
Slope(m)		2684.5	2153.5	
Intercept(c)		484.45	150.3	
Regression equation (Y=mx+c)		y = 2684.5x + 484.45	y = 2153.5x +150.3	
Assay (% mean assay)		99.48%	99.34%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		0.3	0.6	NMT 2.0%
Method precision %RSD		0.3	0.4	NMT 2.0%
Accuracy %recovery		99.29%	99.87%	98-102%
LOD		0.09	0.04	NMT 3
LOQ		0.27	0.13	NMT 10
Robustness	**FM**	0.5	0.5	%RSD NMT 2.0
	FP	0.1	0.2	
	MM	0.2	0.5	
	MP	0.2	0.2	
	TM	0.1	0.3	
	TP	0.1	0.5	

9. CONCLUSION:

For the simultaneous estimate of Sulbactam and Durlobactam in tablet dose form, a straightforward, accurate, and exact approach was established. Sulbactam and Durlobactam were found to have retention times of 2.458 and 2.855 minutes, respectively. Sulbactam and Durlobactam were found to have %RSDs of 0.3 and 0.6, respectively. % For Sulbactam and Durlobactam, recovery rates were 99.29% and 99.87%, respectively. Sulbactam and Durlobactam regression models yielded LOD and LOQ values of 0.09, 0.27, and 0.04, 0.13, respectively. Sulbactam's regression equation is y = 2684.5x + 484.45, while Durlobactam's is y = 2153.5x + 150.3. Because retention times and run times were reduced, the devised approach was straightforward and cost-effective, making it suitable for use in routine quality control testing in industries.

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