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QbD Approach to Hplc Method Development and Validation of the Simultaneous Estimation of Sulbactum and Durlobactum in Pharmaceutical Dosage Form

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INTRODUCTION

The pharmaceutical industry consistently emphasizes the importance of **product quality, safety, and efficacy**. To enhance these aspects, scientific tools such as **Quality by Design (QbD)** and **Process Analytical Technology (PAT)** have been widely implemented. These approaches provide a systematic and science-based framework for pharmaceutical development and manufacturing, aiming to improve product understanding, process control, and ultimately, minimize risks while maximizing productivity and quality.

The QbD framework, initially developed for manufacturing processes, has been successfully extended to the field of **analytical method development**—a concept now termed **Analytical Quality by Design (AQbD)**. AQbD applies the principles of QbD to ensure that analytical methods are not only fit for their intended purpose but also robust and reliable throughout the product lifecycle.

Regulatory bodies, including the **U.S. FDA**, have recognized the importance of QbD and released guidance documents specifically for its implementation in **immediate-release and extended-release formulations**. Moreover, the International Council for Harmonisation (ICH) encourages the application of QbD principles through its guidelines **Q8 to Q11**, supporting its adoption in both formulation and analytical development.

Drug profile:

Sulbactam is a **beta-lactamase inhibitor** commonly used in combination with other antibiotics (like ampicillin) to treat various bacterial infections. It works by blocking the enzyme beta-lactamase, which bacteria use to resist antibiotics. Sulbactam has a molecular formula of **$C_8H_{11}NO_5S$** and a molecular weight of **233.24 Da**. It's available mainly in **injectable form** under brand names such as **Unasyn** and **Sulperazon**. Common side effects include **skin rash, diarrhea, nausea, and vomiting**, and it should be avoided by individuals allergic to beta-lactam antibiotics.

Durlobactam is an **investigational beta-lactamase inhibitor** developed to treat **serious hospital-acquired and ventilator-associated bacterial infections**, especially those caused by resistant strains like **Acinetobacter baumannii**. It has a molecular formula of **$C_8H_{11}N_3O_6S$** and a molecular weight of **277.25 g/mol**. Durlobactam is designed to inhibit a broader range of beta-lactamase enzymes (classes A, C, and D) but is **not active against class B metallo-beta-lactamases**. It is currently not commercially available, and limited clinical data exist regarding its side effects and tolerability.

(R2) Tailing Factor Of Sulbactum And Durlobactum:-

ANOVA for Quadratic model Table 1: Response 2: tailing factor

Source	Squares	df	Square	Value	Prob > F
Model	0.036	9	3.967E-003	9.01	0.0042

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A-Flow rate	5.119E-003	1	5.119E-003	11.62	0.0113
B-Buffer PH	3.178E-004	1	3.178E-004	0.72	0.4237
C-Organic ration MP	8.137E-005	1	8.137E-005	0.18	0.6802
AB	5.167E-003	1	5.167E-003	11.73	0.0111
AC	9.749E-005	1	9.749E-005	0.22	0.6523
BC	1.481E-004	1	1.481E-004	0.34	0.5802
A^2	8.176E-003	1	8.176E-003	18.56	0.0035
B^2	0.014	1	0.014	31.52	0.0008
C^2	4.654E-003	1	4.654E-003	10.57	0.0140
Residual	3.083E-003	7	4.404E-004		
Lack of Fit	3.083E-003	3	1.028E-003		
Pure Error	0.000	4	0.000		
Cor Total	0.039	16			

System Suitability:

Figure 1: Chromatogram for system suitability

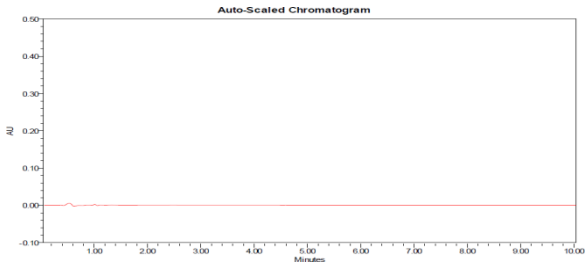

Figure 2: Chromatogram for system suitability

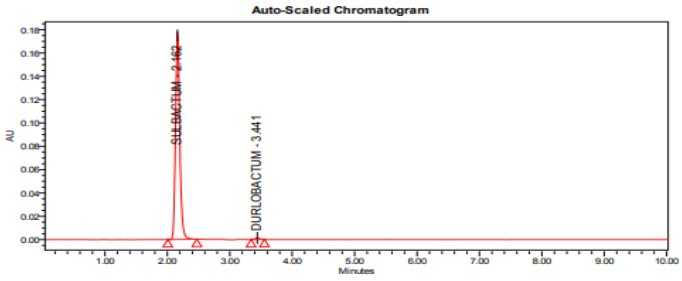

Table 2: Results of system suitability parameters

S.No	Name	RT(min)	Area sec)	Height (µ	USP tailing	USP plate count
1	Sulbactum	2.162	18895	1109	1.0	5797
2	Durlobactum	3.441	789931	22645	0.85	2357

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Acceptance criteria:

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not more than 2.

• It was found from above data that all the system suitability parameters for developed method were within the limit.

Validation Parameters:

ASSAY:

Standard and sample solution injected as described under experimental work. The corresponding chromatograms and results are shown below.

Figure 3: Chromatogram for Standard

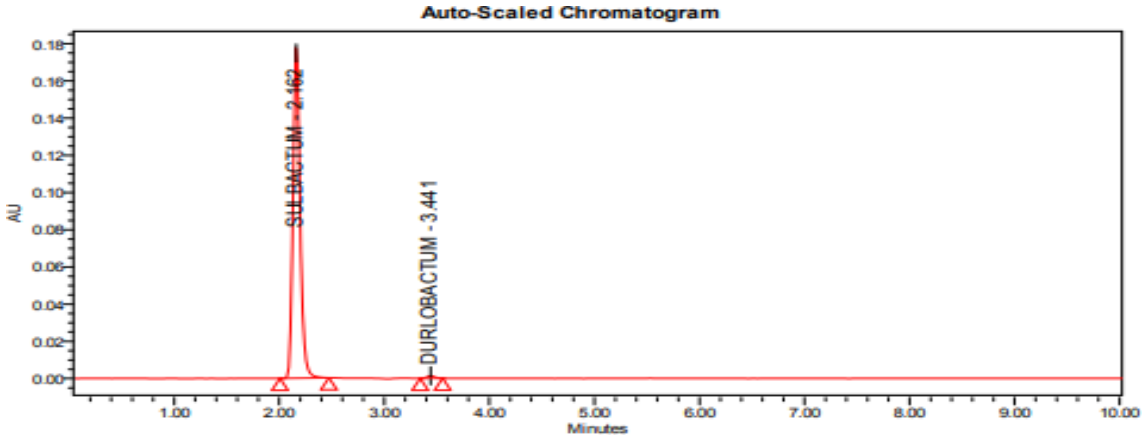

Figure 4: Chromatogram for Sample

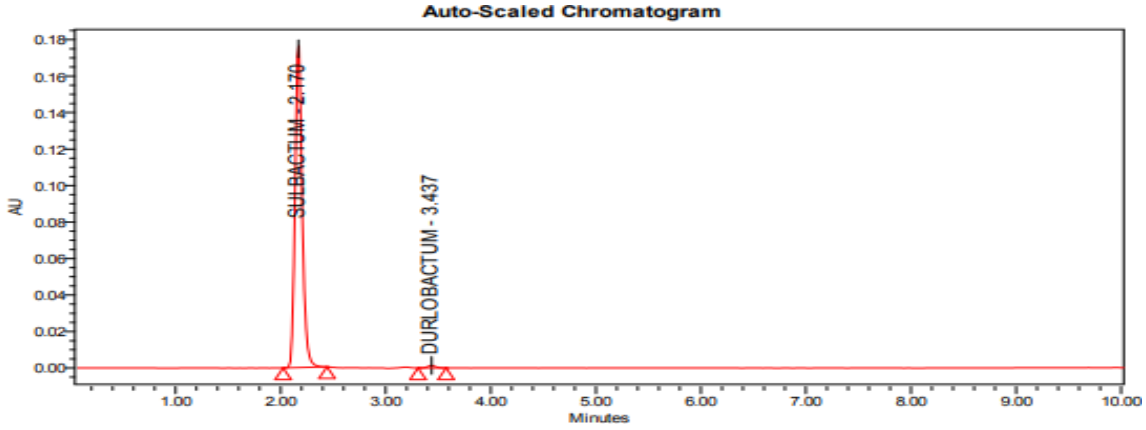

Table:3 Retention Times Of Sulbactum,Durlobactum

S.No	Name(STD)	RT(min)	Area(µV sec)	Height (µV)	USP tailing	USP plate count
1	Sulbactum	2.162	18895	1109	1.0	5797
2	Durlobactum	3.441	789931	22645	0.85	2357

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Table:4 Retention Times Of Sulbactum,Durlobactum

S.No	Name (Sample)	RT(min)	Area(µV sec)	Height (µV)	USP tailing	USP plate count
1	Sulbactum	2.170	18899	1129	1.2	5799
2	Durlobactum	3.437	789938	22695	0.89	2351

Table 5: Results of Assay for Sulbactum and Durlobactum

	Label Claim (mg)	% Assay
Sulbactum and Durlobactum	0.5 g + 1.0 g	101.2

Linearity:

The linearity range was found to lie from 10µg/ml to 50µg/ml of Sulbactum and Durlobactum and chromatograms are shown below

Figure 5: Chromatogram for linearity-1

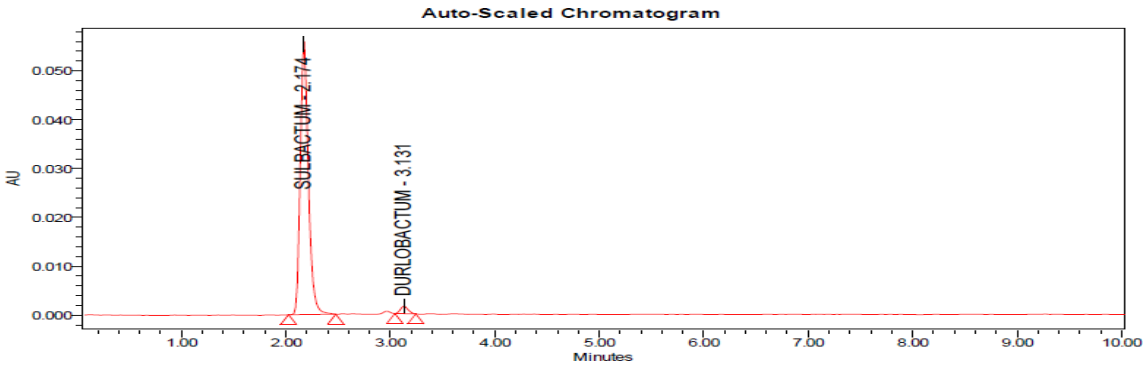

Figure 6 : Chromatogram for linearity-2

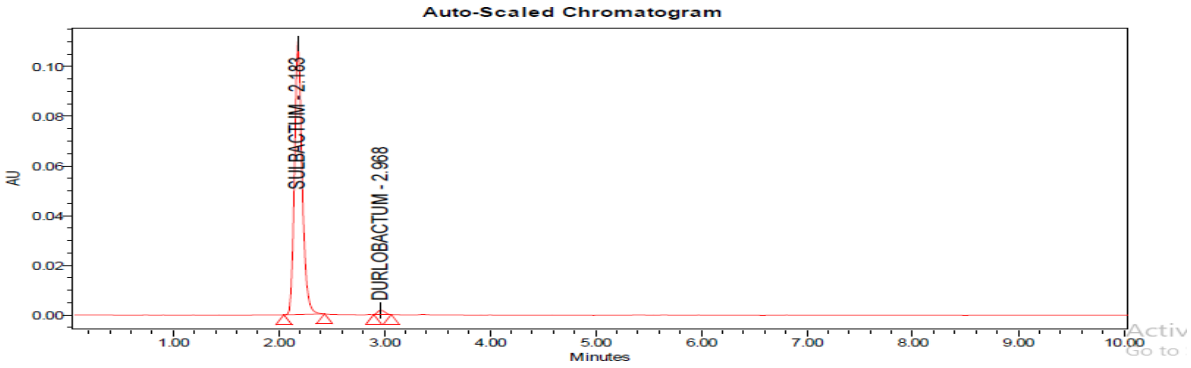

Figure 7: Chromatogram for linearity-3

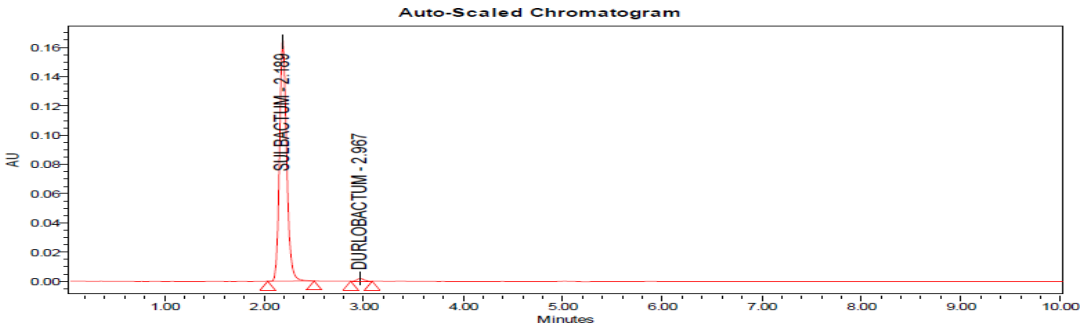

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Figure 8: Chromatogram for linearity-4

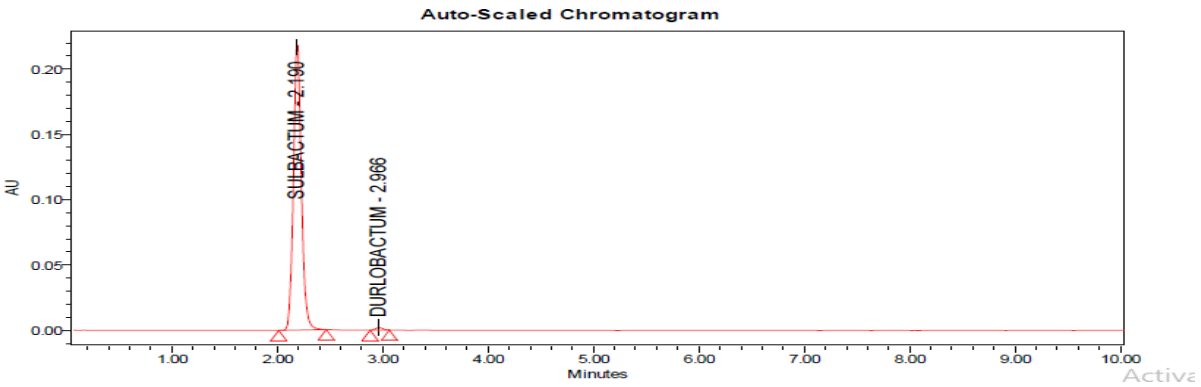

Figure 9: Chromatogram for linearity-5

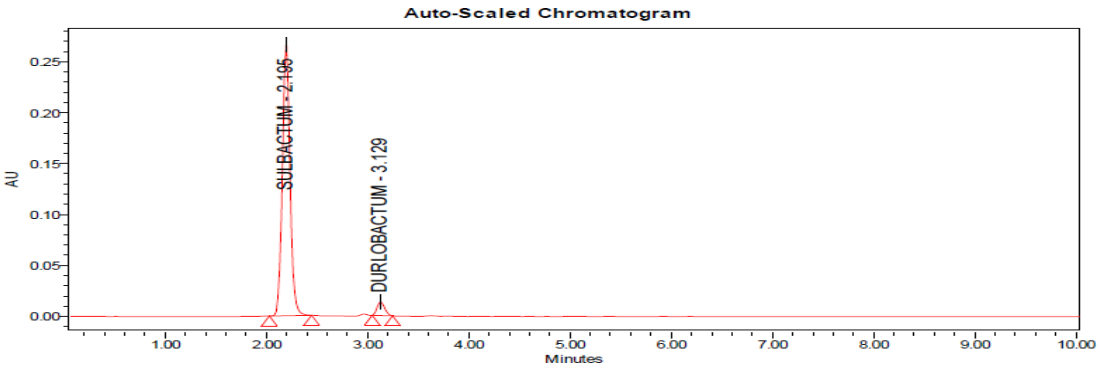

Table 6: Area of different concentration of Sulbactum and Durlobactum

	Concentration (µg/ml) Sulbactum and Durlobactum	Areas Durlobactum	Areas of Sulbactum
1	10	6299	273312
2	20	12599	526625
3	30	18899	789938
4	40	25198	1053250
5	50	32498	1316563

Figure 10: Calibration graph for Sulbactum

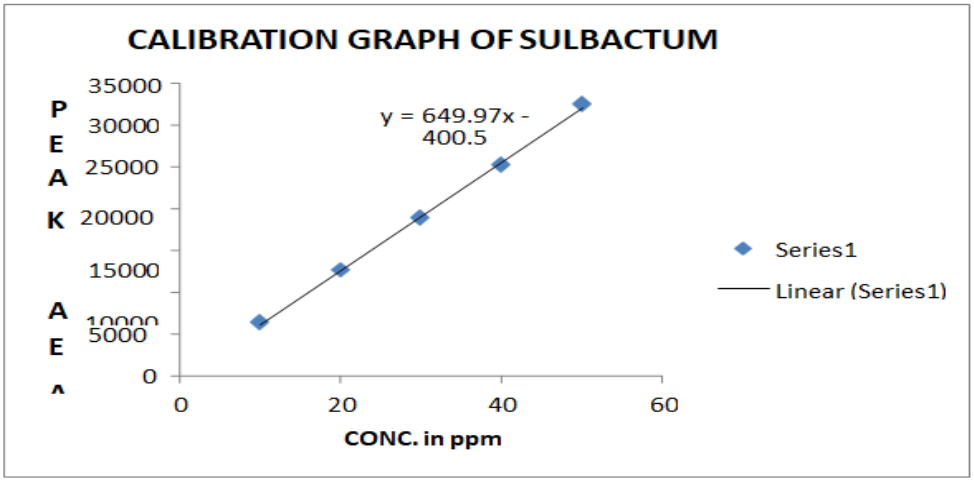

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Figure 11: Calibration graph for Durlobactum

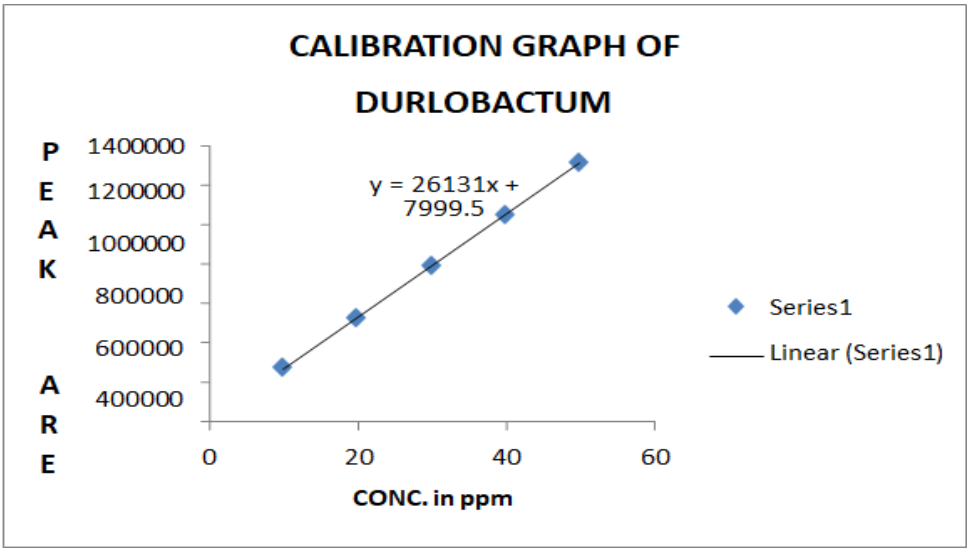

Table 7: Analytical performance parameters of Sulbactum and Durlobactum

Parameters	**Sulbactum**	**Durlobactum**
Slope (m)	649.97	26131
Intercept (c)	400.5	7999.5
Correlation coefficient (R^2)	0.999	0.999

Acceptance criteria:

Correlation coefficient (R^2) should not be less than 0.999

The correlation coefficient obtained was 0.999 which is in the acceptance limit.

Precision:

Precision of the method was carried out for both sample solutions as described under experimental work. The corresponding chromatograms and results are shown below.

Figure 12: Chromatogram for Precision -1

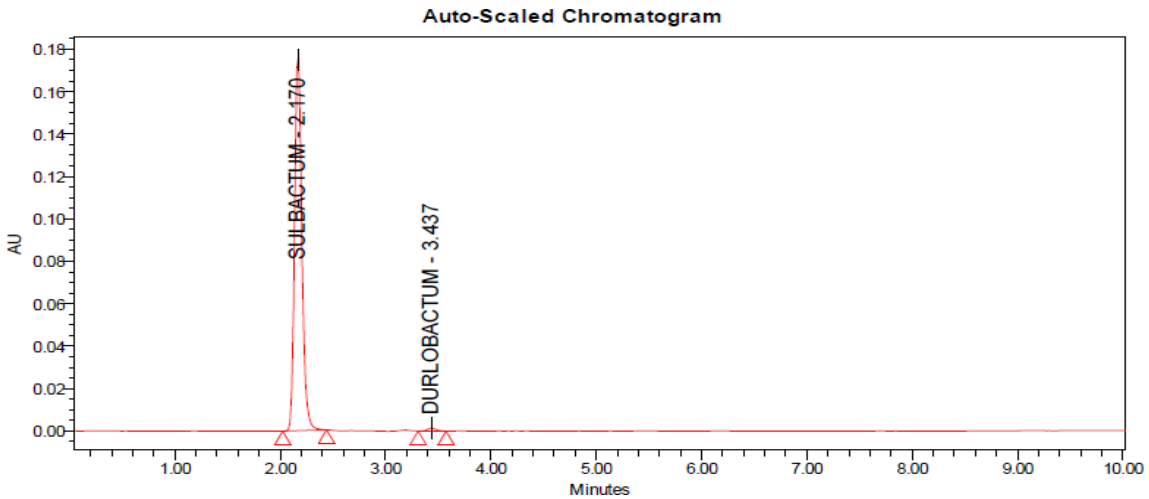

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Figure 13 : Chromatogram for Precision -2

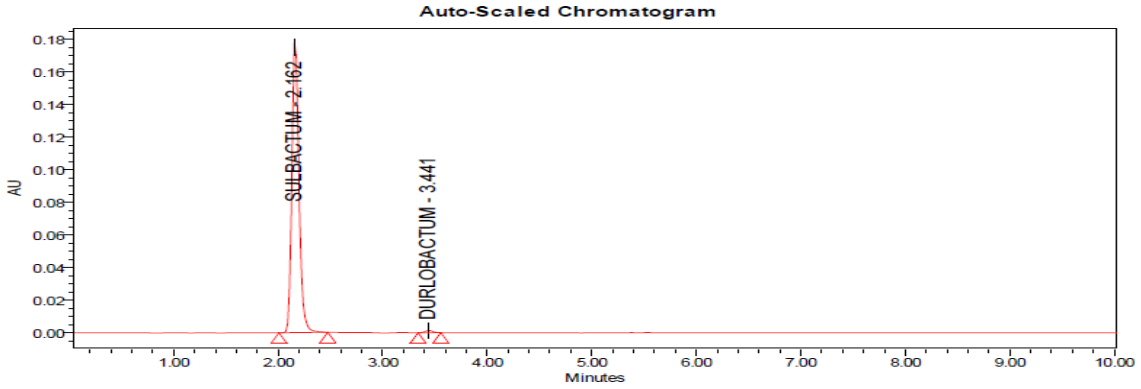

Figure 14: Chromatogram for Precision -3

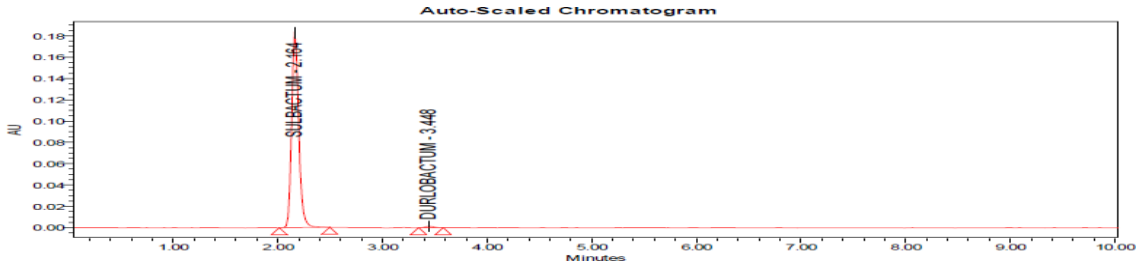

Figure 15: Chromatogram for Precision -4

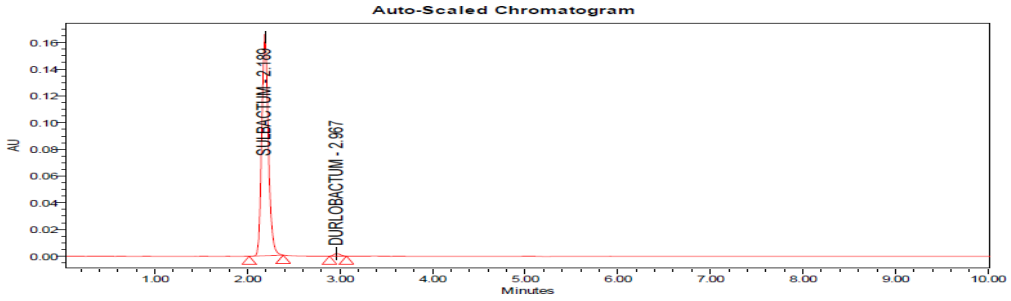

Figure 16: Chromatogram for Precision -5

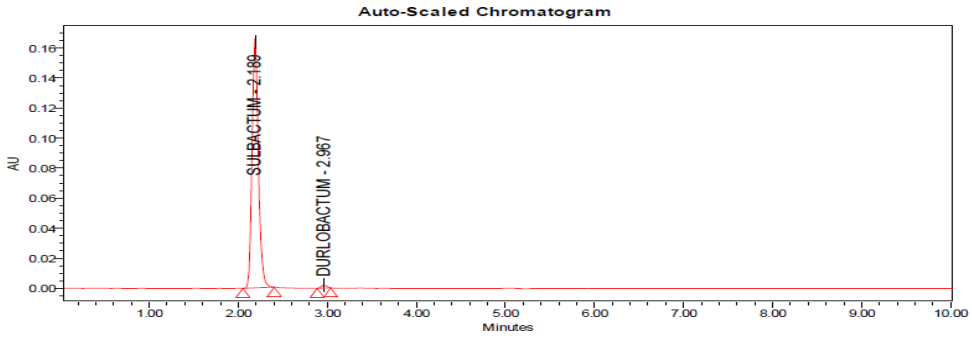

Figure 17: Chromatogram for Precision -6

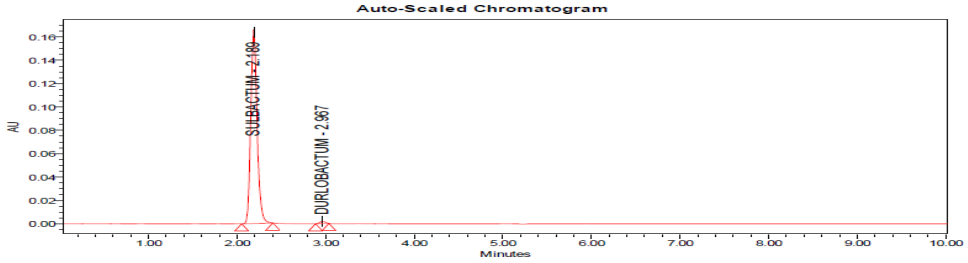

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Table 8: Results of Precision for Sulbactum and Durlobactum

Injection	**Area**	**Area**
Injection-1	7970152	16726
Injection-2	8065041	16157
Injection-3	7899251	16878
Injection-4	7842995	16504
Injection-5	7926488	16948
Injection-6	7951230	16631
Average	7942526	16640.67
Standard Deviation	74679.64	286.6243
%RSD	0.94	1.72

Acceptance criteria:

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate Precision (ruggedness)

There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation.

Figure 18: Chromatogram for ID Precision -1

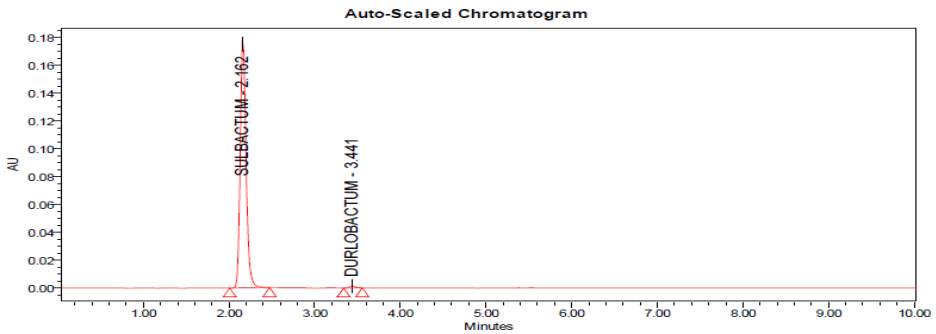

Figure 19: Chromatogram for ID Precision -2

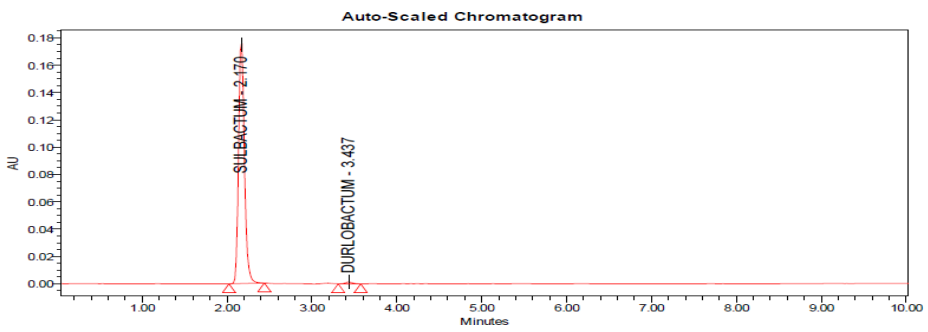

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Figure 20: Chromatogram for ID Precision -3

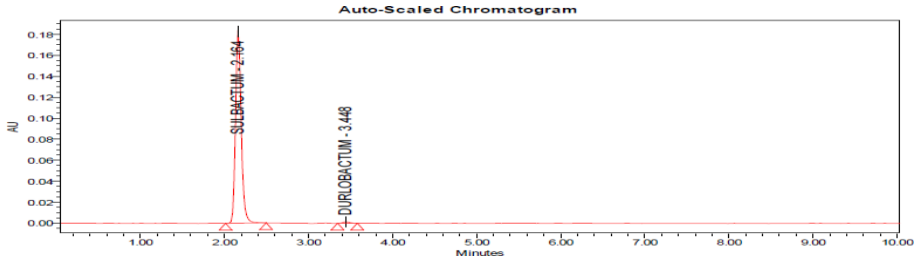

Figure 21: Chromatogram for ID Precision -4

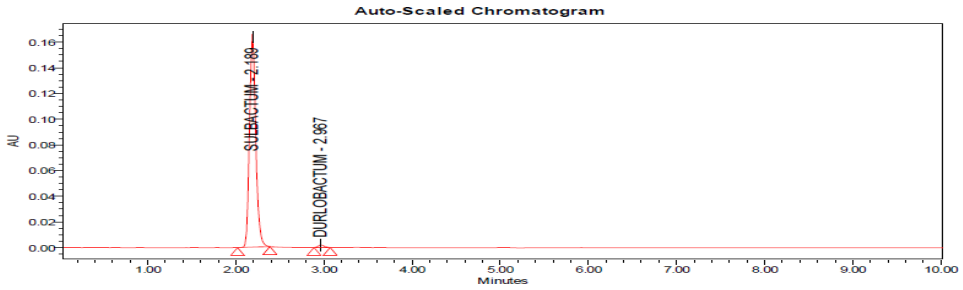

Figure 22: Chromatogram for ID Precision -5

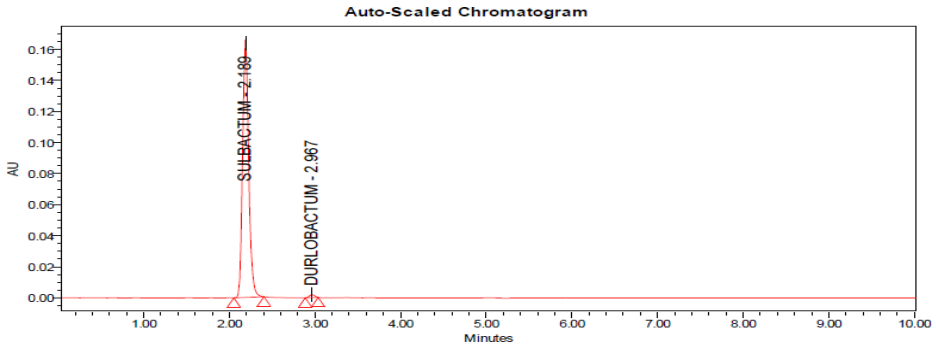

Figure 23: Chromatogram for ID Precision -6

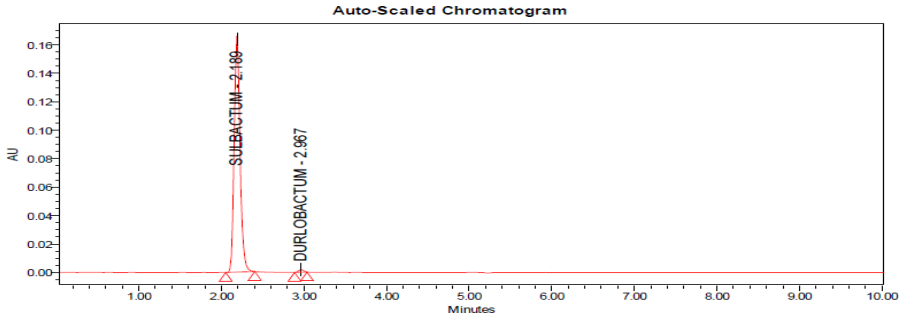

Table 9: Results of Intermediate precision for Sulbactum Durlobactum

Injection	**Area**	**Area**
Injection-1	16726	7970152
Injection-2	16557	8065041
Injection-3	16678	7899251
Injection-4	16514	7842995

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Injection-5	16928	7926488
Injection-6	16631	7951230
Average	**16672.33333**	**7942526**
Standard Deviation	**147.2123183**	**74679.64**
%RSD	**0.8**	**0.9**

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

ACCURACY:

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Figure 24: Chromatogram for Accuracy 50%-1

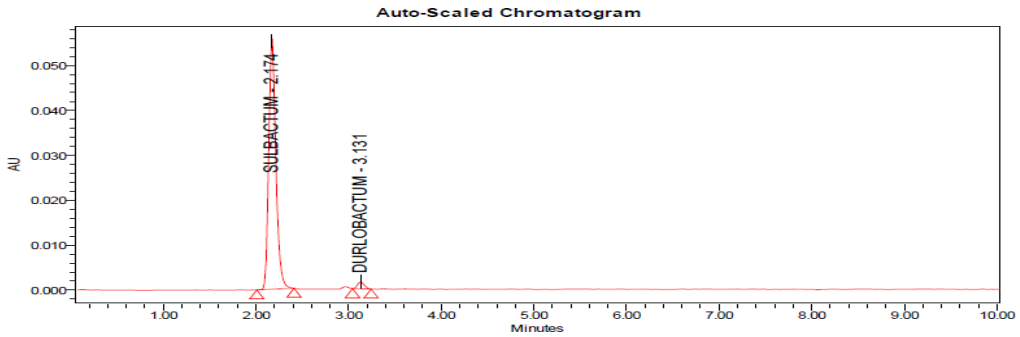

Figure 25: Chromatogram for Accuracy 50%-2

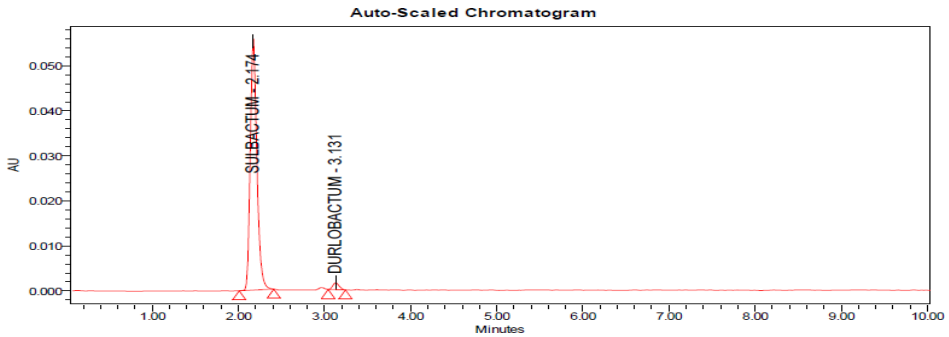

Figure 26: Chromatogram for Accuracy 50%-3

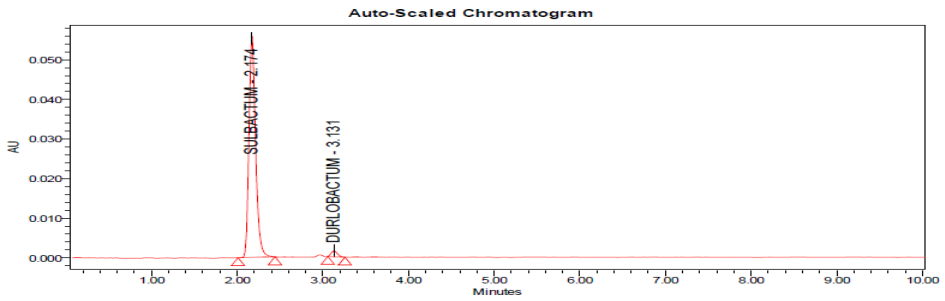

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Figure 27: Chromatogram for Accuracy 100%-1

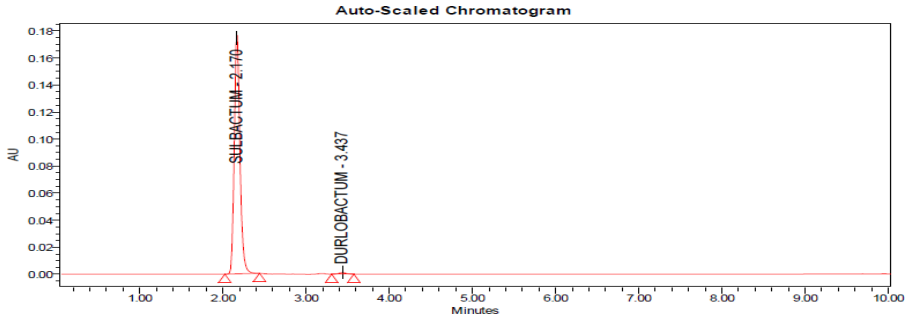

Figure 28: Chromatogram for Accuracy 100%-2

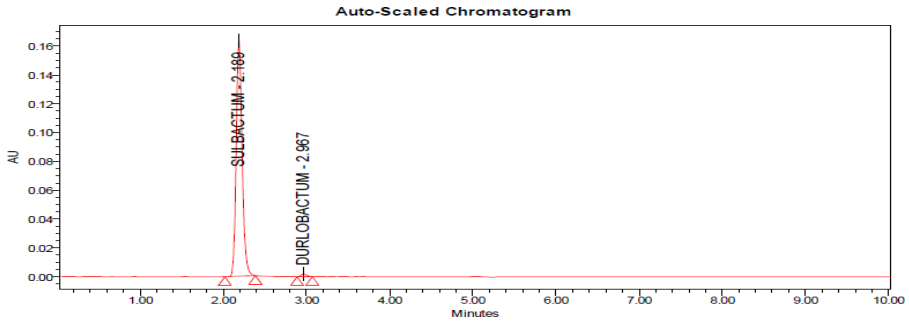

Figure 29: Chromatogram for Accuracy 100%-3

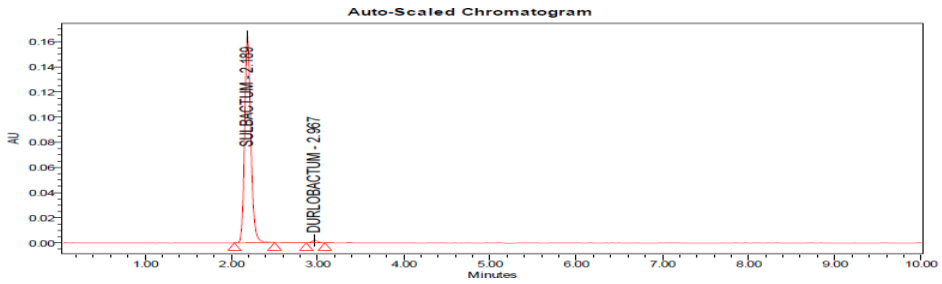

Figure 30: Chromatogram for Accuracy 150%-1

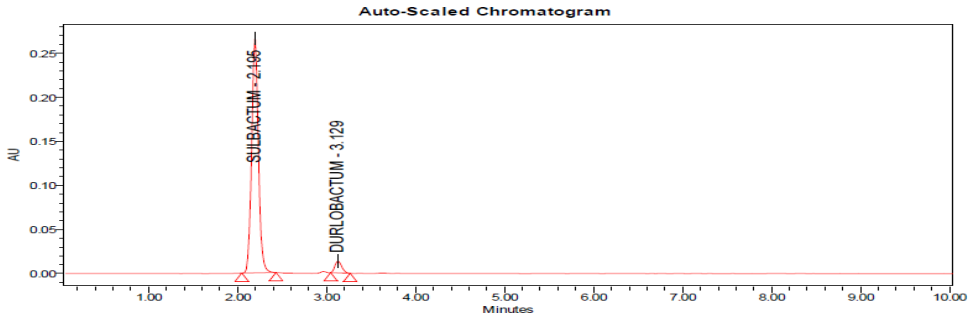

Figure 31: Chromatogram for Accuracy 150%-2

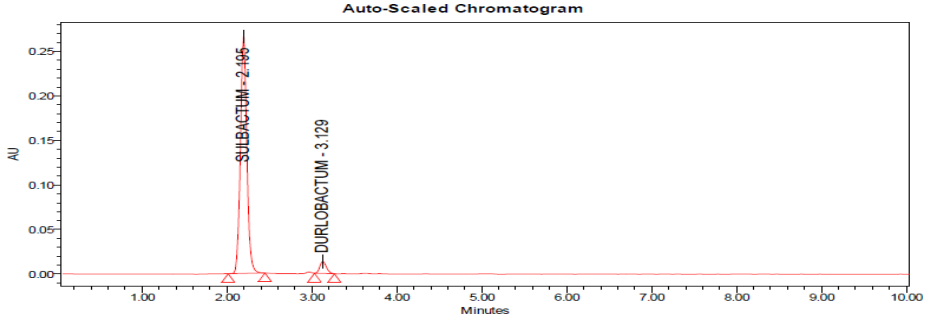

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Figure 32: Chromatogram for Accuracy 150%-3

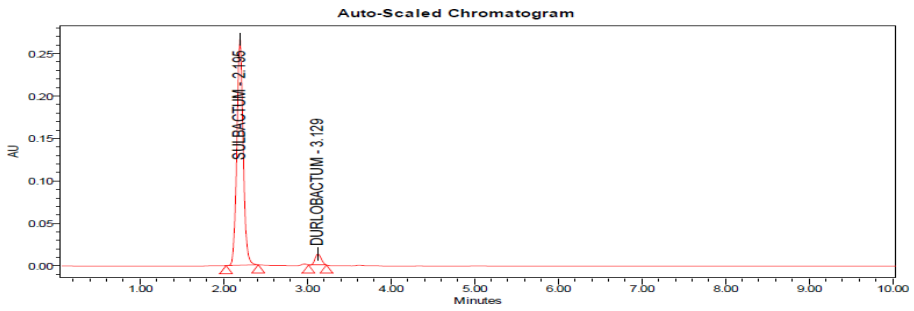

Table 10: Accuracy (recovery) data for Sulbactum and Durlobactum

%Concentration (at specification Level)	Area* Sulbactum	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	9449.5	12.5	12.2	97.6	98.1
100%	18899	25	24.5	98	
150%	28348.5	37.5	37.1	98.9	

%Concentration (at specification Level)	Area* Durlobactum	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	394969	12.5	12.2	97.6	98.1
100%	789938	25	24.5	98	
150%	1184934	37.5	37.1	98.9	

*Average of three determinations

Acceptance Criteria:

The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Limit Of Detection For Sulbactum And Durlobactum

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

Figure 33: Chromatogram of Sulbactum and Durlobactum showing LOD

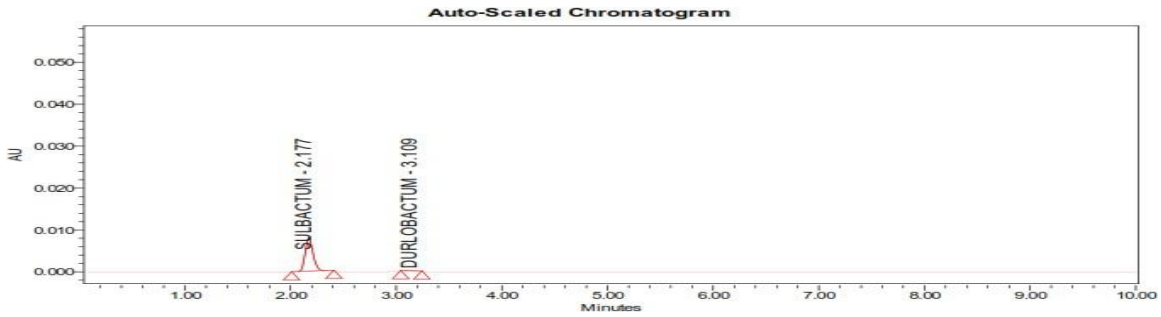

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Table 11: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio	Conc. In ppm
Sulbactum	82	243	2.96	0.38
Durlobactum	75	223	2.97	0.08

- Signal to noise ratio shall be 3 for LOD solution
- The result obtained is within the limit.

Limit Of Quantification For Sulbactum And Durlobactum

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

Figure 34: Chromatogram of Sulbactum and Durlobactum showing LOQ

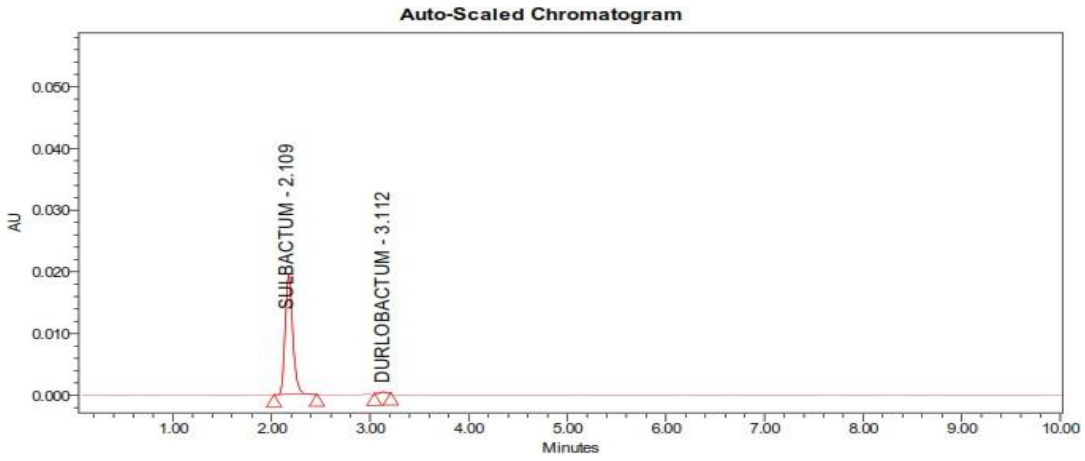

Table 12: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio	Conc. In ppm
Sulbactum	82	818	9.97	1.2
Durlobactum				
Sulbactum Durlobactum	75	745	9.93	0.2

- Signal to noise ratio shall be 10 for LOQ solution
- The result obtained is within the limit.

Robustness:

The standard and samples of Sulbactum and Durlobactumwere injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Variation in flow

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Figure 35: Chromatogram showing less flow

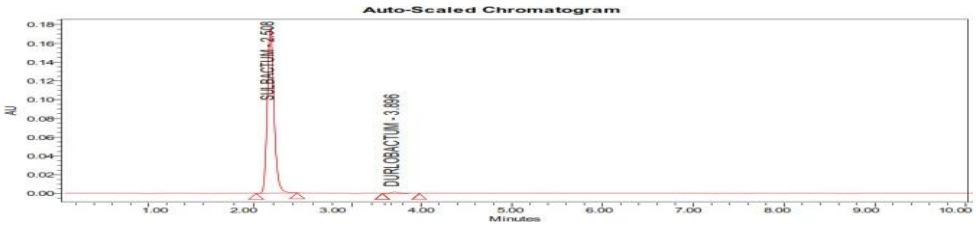
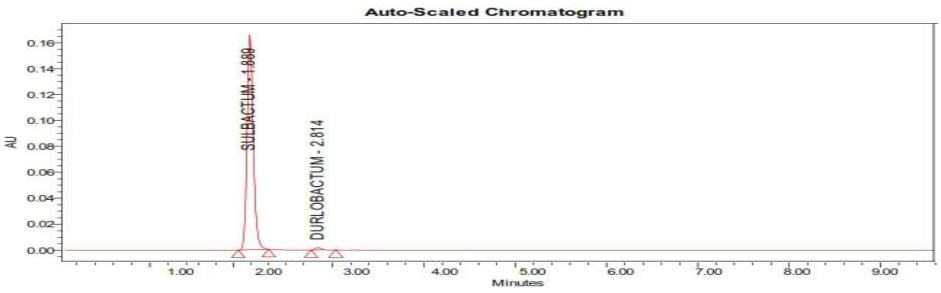


Figure 36: Chromatogram showing more flow



Variation of mobile phase organic composition:

Figure 37: Chromatogram showing less organic composition

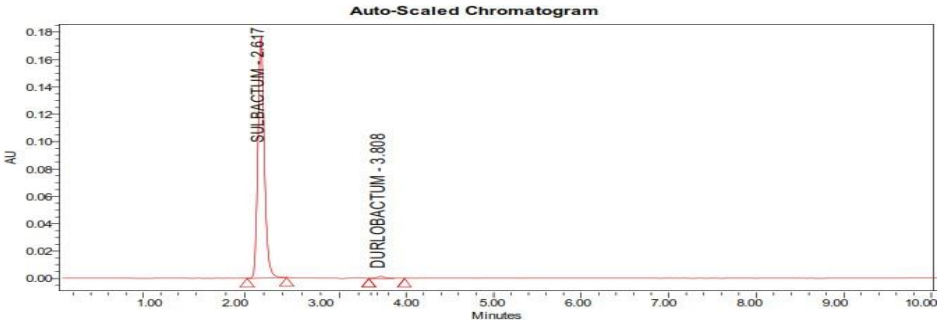


Figure 38: Chromatogram showing more organic composition

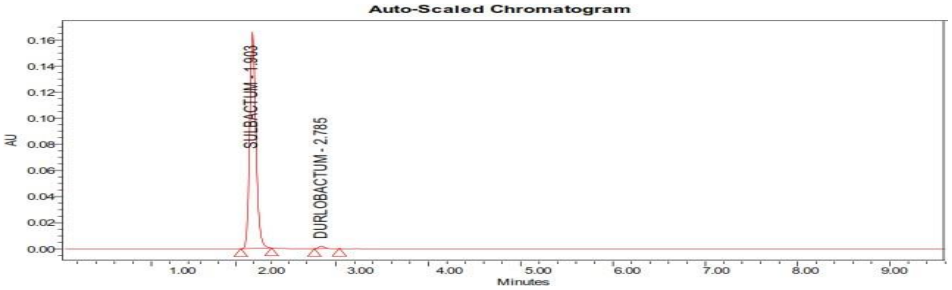


Table 13: Results for variation in flow for Sulbactum and Durlobactum

S. No	Flow (ml/min)	System Suitability Results of Sulbactum		System Suitability Results of Durlobactum	
		USP Plate Count	USP Tailing	USP Plate Count	USP Tailing
1	0.8	5721	1.1	2349	1.01
2	1	5799	1.2	2351	0.89
3	1.2	5793	0.98	2345	0.96

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***** Results for actual flow (1 ml/min) have been considered from Assay standard.

Table 14: Results for variation in mobile phase composition for Sulbactum and Durlobactum

S.	Change in Organic Composition in the Mobile Phase	System Suitability Results of Sulbactum		System Suitability Results of Durlobactum	
		USP Plate Count	USP Tailing	USP Plate Count	USP Tailing
1	10% less	5721	1.1	2349	1.01
2	*Actual	5799	1.2	2351	0.89
3	10% more	5793	0.98	2345	0.96

***** Results for actual Mobile phase composition have been considered from Accuracy standard.

Acceptance criteria:

The Retention time, USP plate count, USP tailing factor obtained for change of flow rate, variation in mobile phase was found to be within the acceptance criteria. Hence the method is robust.

CONCLUSION

The application of QbD principles in the development and validation of the HPLC method for simultaneous estimation of sulbactam and durlobactam proves beneficial in enhancing method reliability and efficiency. By systematically identifying and controlling key variables during method development, the method's robustness and suitability for pharmaceutical analysis are significantly improved.

Furthermore, the establishment of a thorough method validation protocol ensures that the method meets regulatory requirements for accuracy, precision, linearity, specificity, and robustness. The validated method provides a reliable tool for routine analysis of sulbactam and durlobactam in pharmaceutical dosage forms, contributing to the overall quality control and assurance in pharmaceutical manufacturing processes.

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