WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP
Impact Factor 8.63
Volume 15, Issue 12, 1765-1793.
Review Article
ISSN 2277–7105

QBD APPROACH TO HPLC MTHOD DEVELOPMENT AND VALIDATION OF THE SIMULTANEOUS ESTIMATION OF SULBACTUM AND DURLOBACTUM IN PHARMACEUTICAL DOSAGE FORM

[1]P. Ankitha, [1]Intiyaz, [1]B. Unnathi, [1]M. Rubika, [1]Simran Zabeen, [1]Buran Bee, [1]M. Sai Kumar, [1]*K. Susmitha

[1]University College of Pharmaceutical Sciences, Palamuru University, Mahabubnagar, Telangana-509001, India.

Article Received on 15 May 2026,
Article Revised on 05 June 2026,
Article Published on 16 June 2026,

https://doi.org/10.5281/zenodo.20731030

***Corresponding Author**
Dr. K. Susmitha
Asst. Professor, Dept. Of Pharmaceutical Chemistry, University College of Pharmaceutical Sciences, Palamuru University, Mahabubnagar, Telangana-509001, India.

How to cite this Article: *[1]P. Ankitha, [1]Intiyaz, [1]B. Unnathi, [1]M. Rubika, [1]Simran Zabeen, [1]Buran Bee, [1]M. Sai Kumar, [1*]K. Susmitha. (2026). Qbd Approach To Hplc Mthod Development And Validation Of The Simultaneous Estimation Of Sulbactum And Durlobactum In Pharmaceutical Dosage Form. World Journal of Pharmaceutical Research, 15(12), 1765–1793.

This work is licensed under Creative Commons Attribution 4.0 International license.

ABSTRACT

A Quality by Design (QbD)-based RP-HPLC method was developed and validated for the simultaneous estimation of Sulbactam (SUB) and Durlobactam (DUB) in pharmaceutical dosage forms. Critical method parameters were optimized using a Box–Behnken experimental design by evaluating the effects of flow rate, buffer pH, and mobile phase composition on chromatographic performance. Separation was achieved on a Hypersil Gold C18 column (3.0 × 150 mm, 3 µm) using Methanol: 0.1% Formic Acid (65:35, v/v) as the mobile phase at a flow rate of 1.0 mL/min, with UV detection at 220 nm. The retention times of SUB and DUB were found to be 2.162 min and 3.441 min, respectively. The method exhibited excellent linearity over the concentration range of 10–50 µg/mL with correlation coefficients (R²) of 0.999 for both analytes. The assay result was 101.2%, while precision studies showed %RSD values below 2%. Recovery ranged from 97.6% to 98.9%, confirming the accuracy of the method. The developed method was validated according to ICH guidelines and demonstrated satisfactory robustness, sensitivity, and reproducibility. Therefore, the proposed AQbD-based RP-HPLC method is suitable for routine quality control analysis of Sulbactam and Durlobactam in pharmaceutical formulations.

KEYWORDS: Sulbactam, Durlobactam, RP-HPLC, Quality by Design, AQbD, Method Validation.

1. INTRODUCTION

Quality by Design (QbD) has emerged as a scientific and risk-based approach for pharmaceutical development, emphasizing product and process understanding throughout the lifecycle. Regulatory agencies including the International Council for Harmonisation (ICH) recommend QbD implementation through guidelines Q8, Q9, Q10, and Q11[1-4] to ensure consistent product quality. Analytical Quality by Design (AQbD) extends these concepts to analytical method development by systematically identifying analytical target profiles, critical quality attributes, and critical method parameters.

Sulbactam is an irreversible β-lactamase inhibitor used in combination with β-lactam antibiotics to restore antimicrobial activity against resistant pathogens. Durlobactam is a newly developed diazabicyclooctane β-lactamase inhibitor designed to enhance Sulbactam activity against carbapenem-resistant *Acinetobacter baumannii*. Owing to the growing therapeutic importance of this combination, a reliable analytical method capable of simultaneous quantification is essential for quality control and regulatory compliance.

Although several analytical methods have been reported for Sulbactam-containing combinations, no comprehensive AQbD-based RP-HPLC method for simultaneous estimation of Sulbactam and Durlobactam has been extensively reported. Therefore, the present investigation was undertaken to develop and validate a robust AQbD-based RP-HPLC method.[5-7]

2. MATERIALS AND METHODS

Table 1: Instruments used.

SL. No.	Instrument	Model
1	HPLC	WATERS, software: Empower, 2695 separation module.2487 UV detector.
2	UV/VIS spectrophotometer	LABINDIA UV 3000$^+$
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

Table 2: Chemicals used.

SL. No	Chemical	Brand
1	SUB and DUB	Opifex LAB
2	KH_2PO_4	FINARchemicalLTD
3	WaterandMethanolfor HPLC	StandardsolutionsLtd
4	AcetonitrileforHPLC	Standardsolutions Ltd
5	HCL, H_2O_2, NaOH	MERCK

2.1. HPLC METHOD DEVELOPMENT

Wave length selection

SUB and DUB drugs at 10 µg/ml in diluent (MPscomposition) were scanned in the 200–400 nm range to record the UV spectra. From the 220 nm UV spectrum wavelength that was chosen. This wavelength exhibits good absorption for both medications.

Table 3: Development by qbd optimization.

STD	RUN	Block	Factor A. Flow rate	Factor 2 B. Buffer PH	Factor B. Buffer PH	Response 1 Resolution	Response 2 Tailing Factor
4	1	Block 1	1.00	4.50	60.50	4.4	1.03
13	2	Block 1	0.95	4.25	60.50	4.1	1
16	3	Block 1	0.95	4.25	60.50	4.1	1
7	4	Block 1	0.90	4.25	66.00	4	1
15	5	Block 1	0.95	4.25	60.50	4.1	1
8	6	Block 1	1.00	4.25	66.00	4.3	1.02
12	7	Block 1	1.00	4.50	66.00	4.5	1.04
1	8	Block 1	0.90	4.00	60.50	4	1
5	9	Block 1	0.90	4.25	55.00	4	1
6	10	Block 1	1.00	4.25	55.00	4.3	1.02
14	11	Block 1	0.95	4.25	60.50	4.1	1
3	12	Block 1	0.90	4.50	60.50	4	0.9
10	13	Block 1	0.95	4.50	55.00	4	0.9
2	14	Block 1	1.00	4.00	60.50	4.2	1.01
9	15	Block 1	0.95	4.00	55.00	4	0.9
17	16	Block 1	0.95	4.25	60.50	4.1	1
11	17	Block 1	0.95	4.00	66.00	4	0.9

Table 4: Software information.

FileVersion	22.0.4.0		
StudyType	ResponseSurface	**Subtype**	Randomized
Design Type	Box-Behnken	**Runs**	17
DesignModel	Quadratic	**Blocks**	NoBlocks
BuildTime(ms)	4.00		

Table 5: Factors.

Factor	**Name**	**Units**	**Type**	**Low Actual**	**High Actual**	**Low Coded**	**High Coded**	**Mean**	**Std. Dev.**
A	Flow rate	ml/min	Numeric	0.90	1.00	-1.000	1.000	0.953	0.036
B	Buffer PH		Numeric	4.00	4.50	-1.000	1.000	4.250	0.171
C	Organic ration MP	ml	Numeric	55.00	66.00	-1.000	1.000	60.500	3.773

Table 6: Responses.

Factor	**Name**	**Units**	**Type**	**Low Actual**	**High Actual**	**Low Coded**	**High Coded**	**Mean**
Y1	Resolution		17	Polynomial	4.00	4.50	4.13	0.15
Y2	Tailing		17	Polynomial	0.90	1.04	0.98	0.048

Optimization of Column

Hypersil Gold C18 (3.0 x 150mm, 3µm) was found to be ideal as it givefine peak shape and resolution at 1.0 ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used : HPLC and PDA detector

Column : Hypersil Gold C18 (3.0 x 150mm, 3µm)

Mobile phase : 65% Methanol: 35% Formic acid

Flow rate : 1ml/min

Wavelength: 220 nm

Injection volume : 10 µl

Run time : 10 min.

PREPARATION OF BUFFER AND MOBILE PHASE

Preparation of 0.1% Formic Acid at pH 3.5

To create a formic acid solution, add 0.1 ml of formic acid to 1000 ml of water. Adjust the pH of this solution to 3.5 using sodium hydroxide.

Preparation of Mobile Phase

Combine 650 ml of methanol (65%) with 350 ml of formic acid (35%) and degas the mixture in an ultrasonic water bath for 5 minutes. Subsequently, filter the solution through a 0.45 µm filter using vacuum filtration.

Diluent Preparation

Prepare a diluent with a ratio of methanol to 0.1% formic acid at 650:350 ml.

System Suitability

The tailing factor for the peaks corresponding to SUB and DUB in the standard solution should not exceed 2.0. Additionally, the theoretical plates for the SUB and DUB peaks in the standard solution must be no less than 2000.Calculation: (For SUB and DUB).

$$\% \, Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\, weight}{Label\, Claim} * \frac{P}{100} * 100$$

Where: AT= average area counts of sample preparation; AS = average area counts of standard preparation; WS= Weight of working standard taken in mg; P = Percentage purity of working standard; LC = Label Claim mg/ml.

1) Tailing factor Obtained from the standard injection is 1.16.
2) Theoretical Plates Obtained from the standard injection is 3338.

2.2. VALIDATION PARAMETERS

1. ASSAY

Standard Solution Preparation

Weigh 25 mg of the working standards SUB and DUB with precision and transfer them into a fresh, dried out 25 ml volumetric flask. Add the appropriate diluents and sonicate the mixture until fully dissolved. Adjust the volume to the calibration mark with the same solvent to create the stock solution. Next, pipette 0.3 ml from each of the stock solutions into two separate 10 ml volumetric flasks and dilute to the mark with diluents.[8]

Sample Solution Preparation

Transfer the sample's equivalent weight of DUB and 25 mg of SUB into a dry, clean 25 ml volumetric flask. After adding around 7 ml of diluents, sonicate the mixture until the sample dissolves entirely. To prepare the stock solution, bring the volume up to the mark using the same solvent. Then, using diluents, dilute to the appropriate volume in two 10-milliliter volumetric flasks by pipetting 0.3 millilitres from each of the stock solutions.[9-11]

Procedure

Inject 10 µL of both the standard and sample into the chromatographic system. Measure the areas of the SUB and DUB peaks, and calculate the percentage assay using the appropriate formula.

2. LINEARITY

Preparation of Stock Solution

Weigh 25 mg of the SUB and DUB working standards with precision and transfer them into two separate 25 ml clean, dry volumetric flasks. Add the appropriate diluents and sonicate until fully dissolved. Adjust the volume to the mark with the same solvent to create the stock solution.

Preparation of Level I (10 ppm of SUB and DUB): Transfer 0.1 ml of the stock solution + makeup 10 ml volumetric flask with diluents.

Preparation of Level II (20 ppm of SUB and DUB): Transfer 0.2 ml of the stock solution.
Preparation of Level III (30 ppm of SUB and DUB): Transfer 0.3 ml of the stock solution.
Preparation of Level IV (40 ppm of SUB and DUB): Transfer 0.4 ml of the stock solution.
Preparation of Level V (50 ppm of SUB and DUB): Transfer 0.5 ml of the stock solution

Procedure

Inject each Con.c level into the chromatographic system and record the peak area. Create a graph plotting peak area against Con.c (Con.c on the X-axis and peak area on the Y-axis) and calculate the correlation coefficient.

3. PRECISION

Stock preparation Solution

Transfer 25 mg of the SUB and DUB working standard into a 25 ml dry volumetric flask after carefully weighing it. To thoroughly dissolve it, add diluents and sonicate. To raise the volume to the necessary level, use the same solvent. Pipette an extra 0.3 ml of the previously indicated stock solution into a 10-milliliter volumetric flask. Dilute with diluents to the necessary amount.

Procedure

Six injections of the standard solution were made, and each injection's area was measured in an HPLC. It was discovered that the %RSD for the region of six duplicate injections fell between the required bounds.

4. INTERMEDIATE PRECISION/RUGGEDNESS

To assess the intermediate precision, commonly referred to as Ruggedness, of the method, precision tests were conducted on different days within the laboratory.

Preparation of the stock solution

Accurately weigh out 25 mg of the SUB and DUB working standards, and then pour them into a dry, clean 25 ml volumetric flask. To make the stock solution, add the diluents and sonicate until completely dissolved. Then, use the same solvent to raise the volume to the appropriate level.

After that, pipette 0.3 ml of the stock solution into a 10-milliliter volumetric flask and use the diluents to dilute it to the appropriate level.

Procedure

The standard solution was injected six times in HPLC. The %RSD for the areas of the six replicate injections was determined to be within the acceptable limits.

5. ACCURACY

To determine accuracy, three distinct Con.c s were prepared separately: 50%, 100%, and 150% for the analyte, with corresponding Chromatograms (CG)s recorded for each.

Preparation of Standard Stock Solution

Adopted above the method (Ruggedness).

Preparation of Sample Solutions

For the 50% solution, 12.5 mg of the working standards SUB and DUB should be precisely weighed and transferred into a dry, clean 25 ml volumetric flask. To create the stock solution, add the diluent, sonicate to guarantee full dissolution, and then adjust the volume to the mark using the same solvent. Then, use the diluent to dilute 0.3 ml of this stock solution to the appropriate volume in a 10 ml volumetric flask.

For the 100% solution, 25 mg of the working standards SUB and DUB should be weighed and transferred into a dry, clean 25 ml volumetric flask. To prepare the stock solution, add the diluent and sonicate until completely dissolved. Then, use the same solvent to make up the remaining volume. Pipette 0.3 ml of the stock solution into a 10 ml volumetric flask once again, and then use the diluent to dilute it to the appropriate level.

For the 150% solution, 37.5 mg of the working standards SUB and DUB should be weighed and transferred into a dry, clean 25 ml volumetric flask. To guarantee total dissolution, add the diluent and sonicate. Next, use the same solvent to raise the volume to the appropriate level and create the stock solution. Last but not least, pipette 0.3 ml of this stock solution into a 10 ml volumetric flask and use the diluent to dilute it to the appropriate level.

Procedure

Inject the standard solutions for Accuracy -50%, Accuracy -100%, and Accuracy -150%. Calculate the amount found and the amount added for SUB and DUB, and perform the necessary calculations.

6. LOD

Preparation of SUB and DUBsolution

Making the 0.4 µg/ml solution

Weigh precisely 25 mg of SUB and DUB working standard, transfer them to a 25 ml dry volumetric flask, add diluents, and sonicate to ensure complete dissolution. To get the volume to the right level, use the same solvent. (Assembly of stocks).

After pipetting the previously described stock solution into a 10 ml volumetric flask, dilute it to the necessary amount using diluents.

After pipetting the previously described stock solution into a 10 ml volumetric flask, dilute it to the necessary amount using diluents.

The stock solution indicated earlier should be diluted with 0.4 ppm of diluent and then added to a 10 ml volumetric flask.

7. LOQ

Preparation of SUB and DUBsolution[12-13]

Making the 1.3µg/ml solution

To ensure full dissolution, weigh precisely 25 mg of SUB and DUBworking standard, place them into a 25 ml dry volumetric flask, add diluents, and sonicate. Use the same solvent to adjust the volume to the desired level. (Stock arrangement).

The stock solution mentioned above should then be pipetted into a 10 ml volumetric flask and diluted with diluents to the appropriate level.

The stock solution mentioned above should then be pipetted into a 10 ml volumetric flask and diluted with diluents to the appropriate level.

In a 10 ml volumetric flask, pipette an additional 1.3 ml of the stock solution mentioned above, then dilute with diluent to the required level.(1.3 ppm).

8. ROBUSTNESS

Deliberate changes were made to the Flow rate, MPscomposition, and Temperature Variation as part of the Robustness to assess the effect on the procedure.

a) A range of 0.8 to 1.2 ml/min was observed in the flow rate.

A standard solution containing 30 µg/ml of both SUB and DUB was produced and analysed, use both method flow rate and variable flow rates.

b) There was a variation in the Mobile phase's Organic content from 40% to 60%.

In addition to the actual MPscomposition in the procedure, a standard solution containing 30 µg/ml of SUB and DUB was produced and analysed using the changed MPscomposition.

3. RESULTS AND DISCUSSION

Response-1 RESOLUTION OF SUB AND DUB

Table 7: Fit summary.

Source	Sum of Squares	df	Mean Square	F Value	Prob > F	
Mean vs Total	289.88	1	289.88			
Linear vs Mean	0.32	3	0.11	19.93	< 0.0001	
2FI vs Linear	0.041	3	0.014	4.60	0.0286	
Quadratic vs 2FI	0.024	3	7.985E-003	9.76	0.0068	Suggested
Cubic vs Quadratic	5.725E-003	3	1.908E-003	6.366E+007	< 0.0001	Aliased
Residual	0.000	4	0.000			
Total	290.28	17	17.08			

ANOVA for Quadratic model

Table 8: Response 1: Resolution.

Source	**Sum of Squares**		**Mean Square**	**F-value**	**p-value**	
Model	0.39	9	0.043	52.93	< 0.0001	significant
A-MPs Ratio	0.19	1	0.19	238.34	< 0.0001	
B-Flow Rate	0.018	1	0.018	22.07	0.0022	
C-Buffer pH	4.519E-003	1	4.519E-003	5.53	0.0510	
AB	0.014	1	0.014	17.07	0.0044	
AC	2.194E-004	1	2.194E-004	0.27	0.6205	
BC	8.225E-003	1	8.225E-003	10.06	0.0157	
A^2	0.023	1	0.023	28.21	0.0011	
B^2	2.524E-003	1	2.524E-003	3.09	0.1224	
C^2	2.524E-003	1	2.524E-003	3.09	0.1224	
Residual	5.725E-003	7	8.178E-004			

Lack of Fit	5.725E-003	3	1.908E-003			
Pure Error	0.000	4	0.000			
Cor Total	0.40	16				

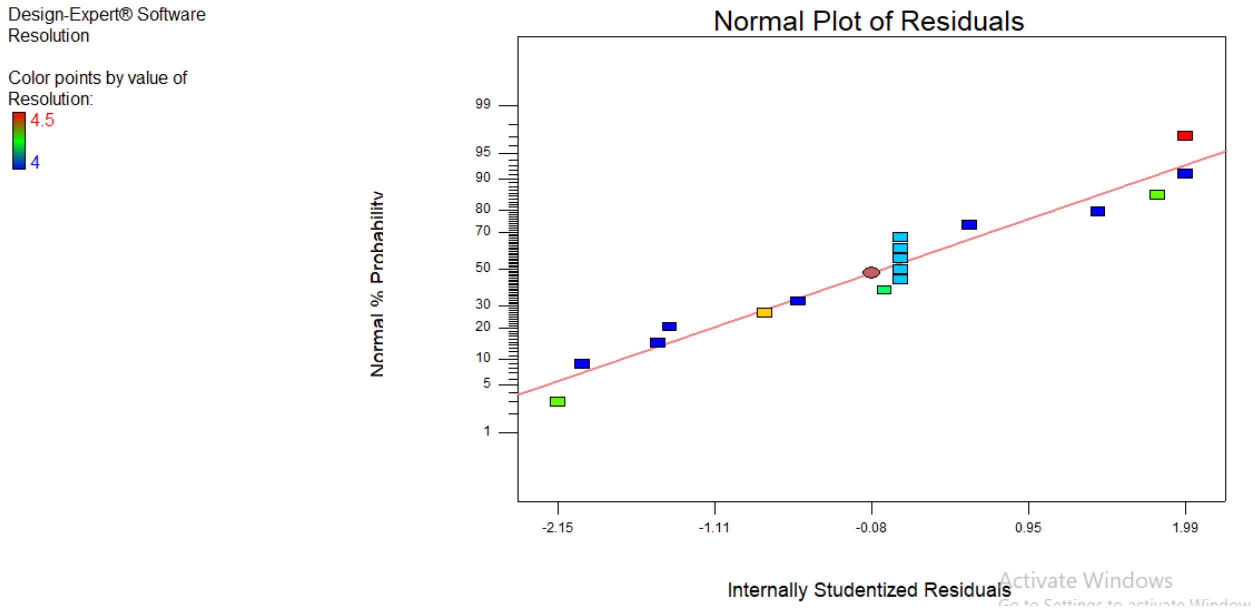

Figure 1: Residuals for SUB and DUB.

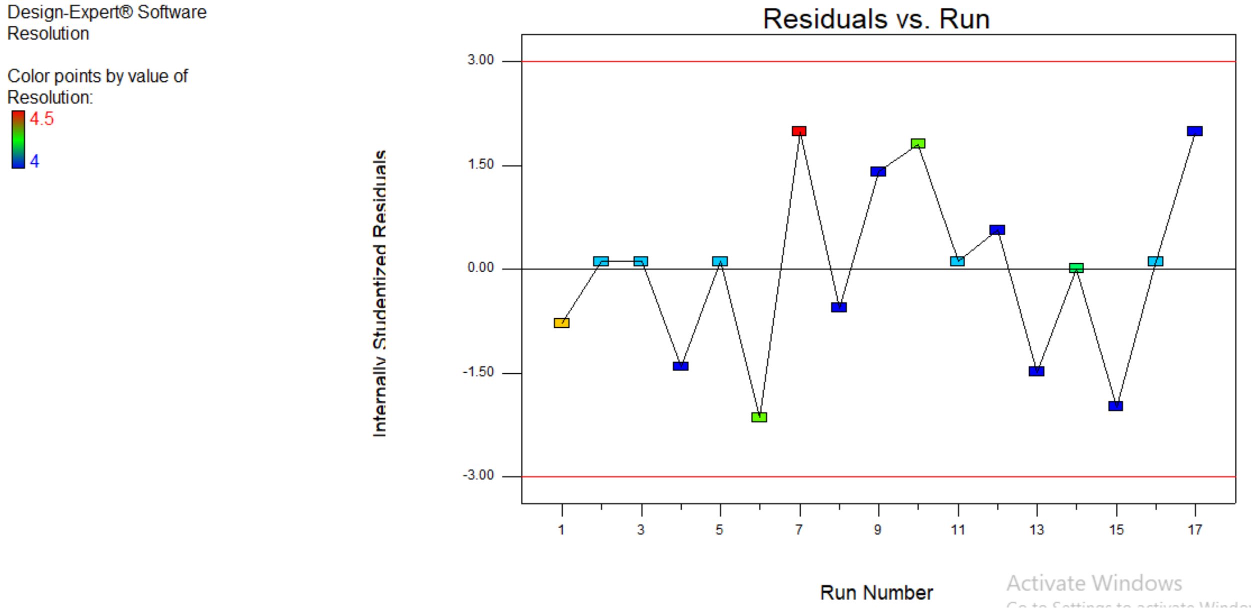

Figure 2: Residuals vs. Run for SUB and DUB.

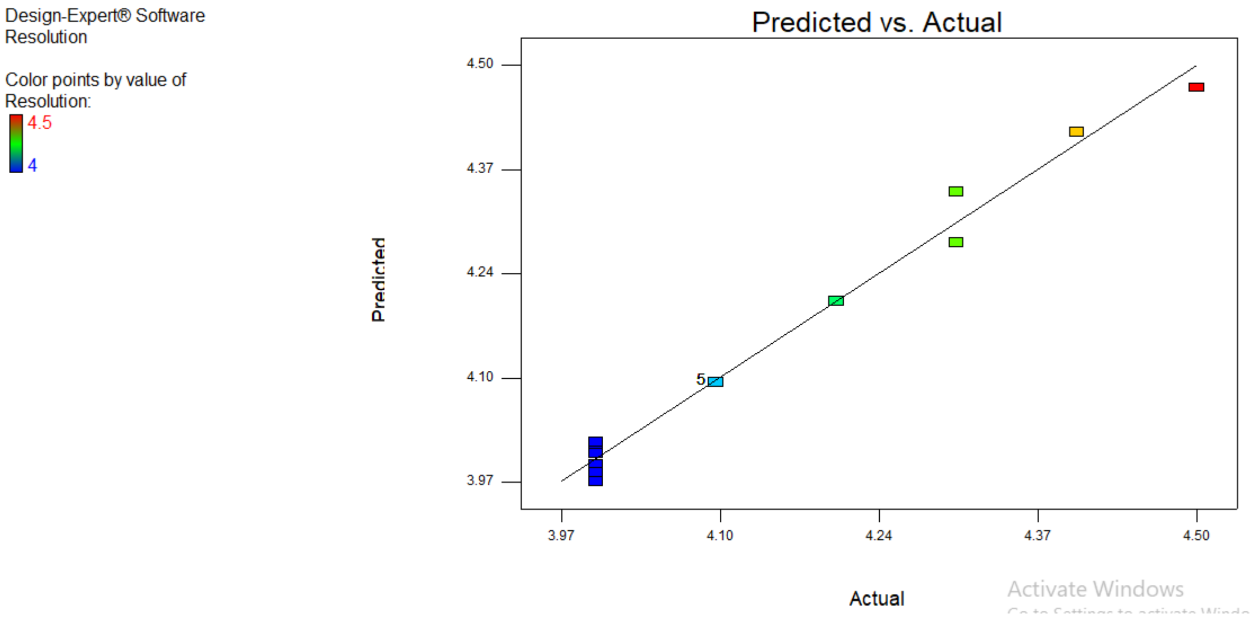

Figure 3: Predicted vs. Actual for SUB and DUB.

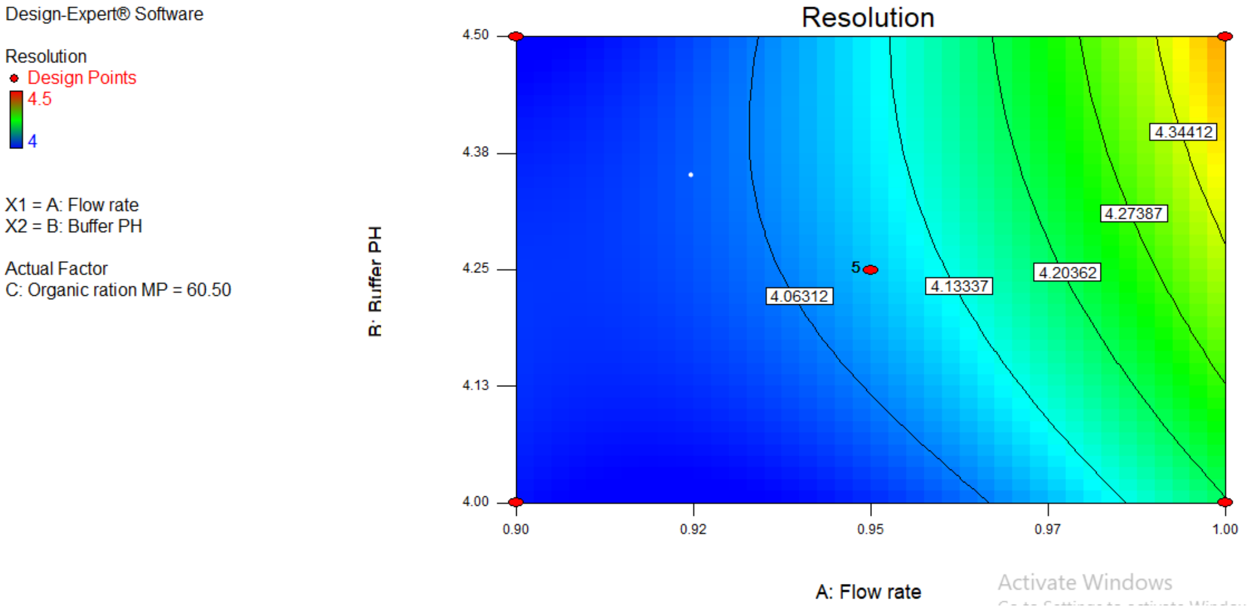

Figure 4: Retention time for SUB and DUB.

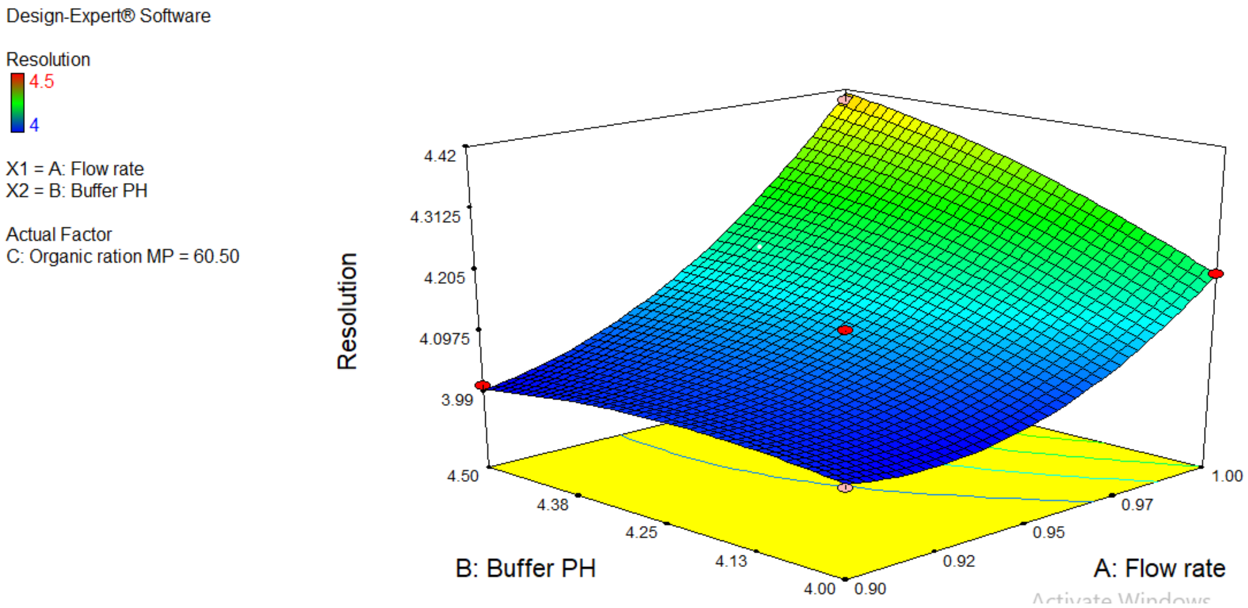

Figure 5: 3D Surface for SUB and DUB.

(R_2) TAILING FACTOR OF SUB AND DUB

ANOVA for Quadratic model

Table 9: Response 2: tailing factor.

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

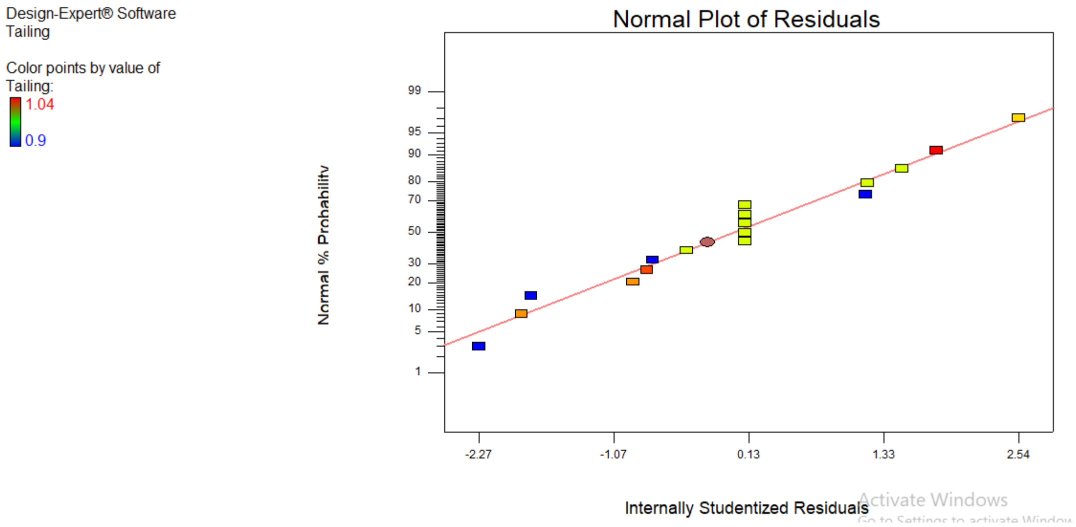

Figure 6: Residuals for SUB and DUB.

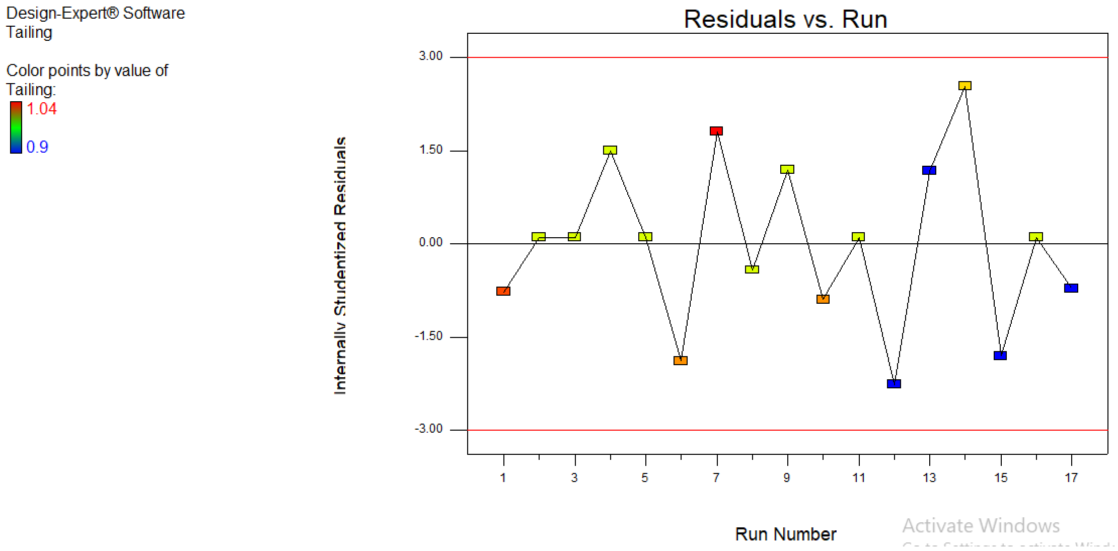

Figure 7: Residuals vs .Run for SUB and DUB.

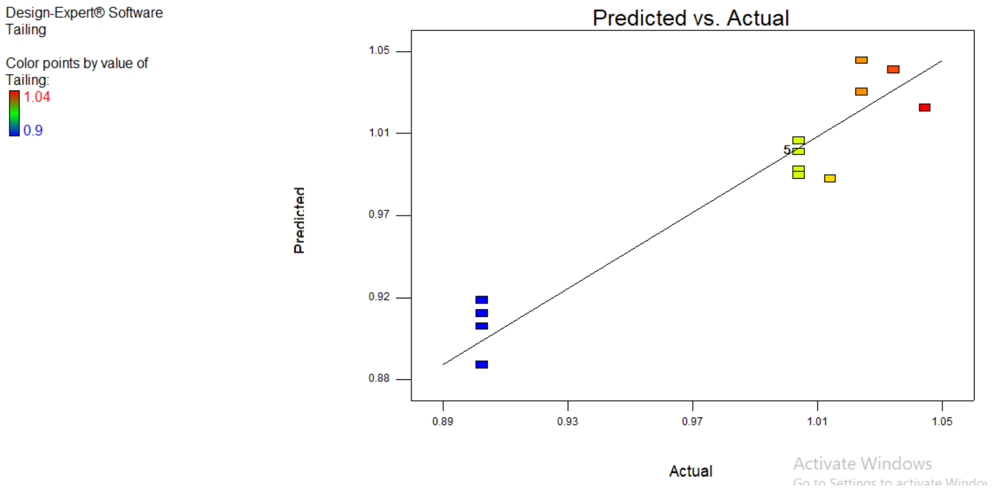

Figure 13: Predicted vs. Actual for SUB and DUB.

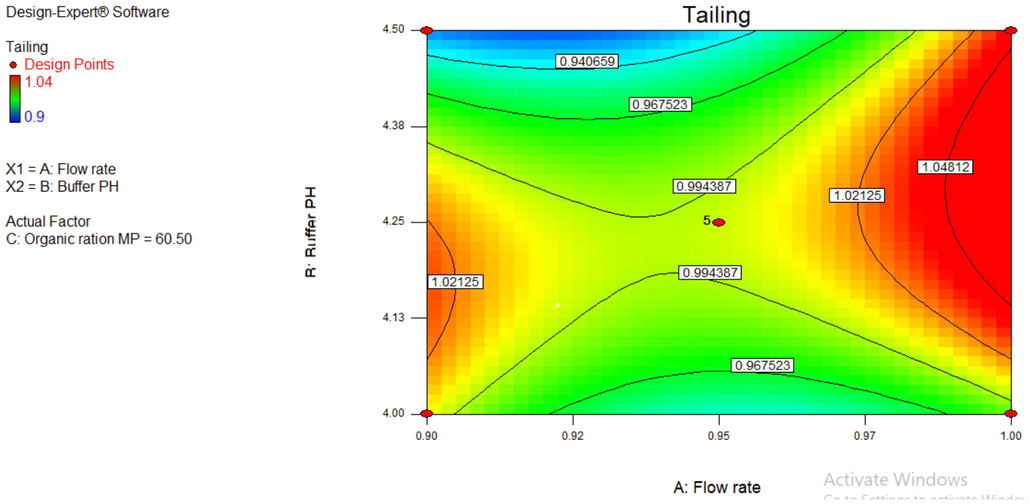

Figure 8: R tailing factor for SUB and DUB.

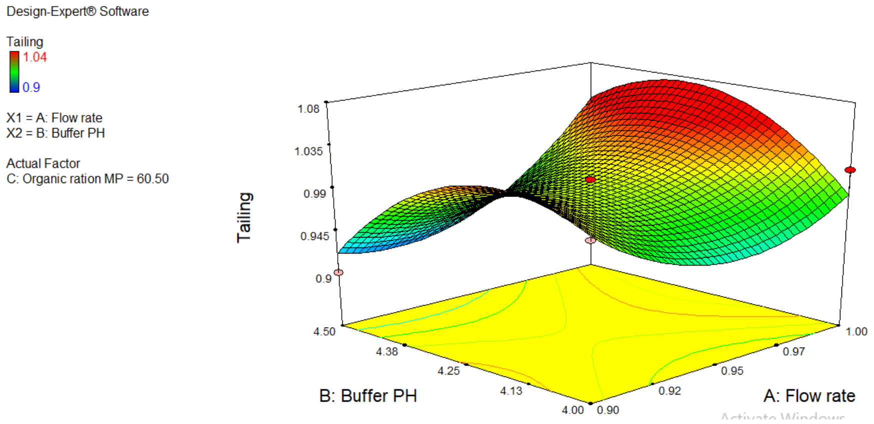

Figure 9: 3D Surface for SUB and DUB.

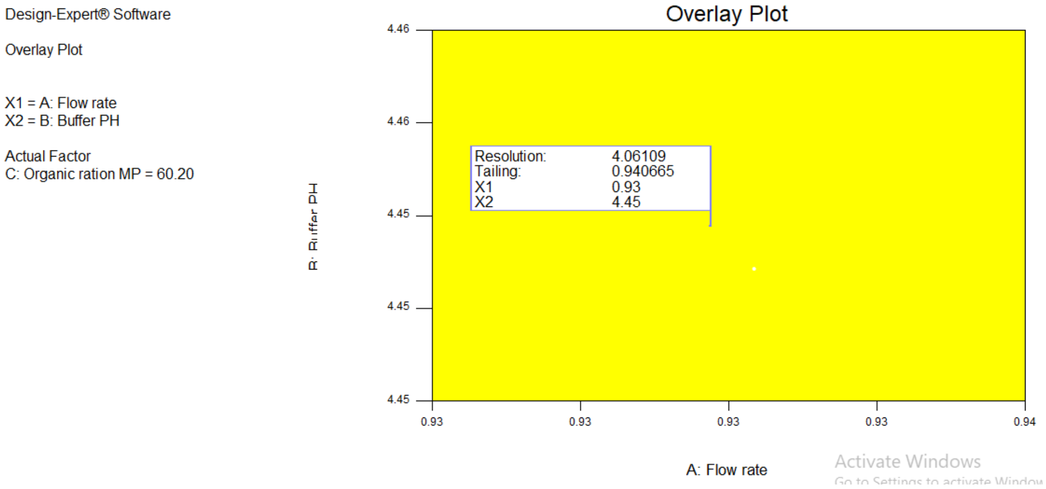

Figure 10: Overlay plot for SUB and DUB.

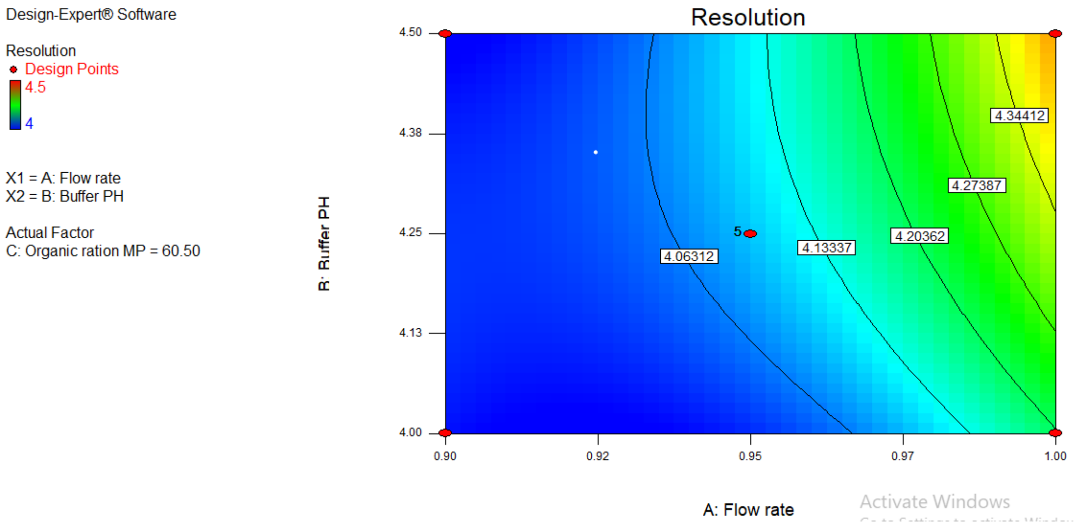

Figure 11: Retention Time for SUB and DUB.

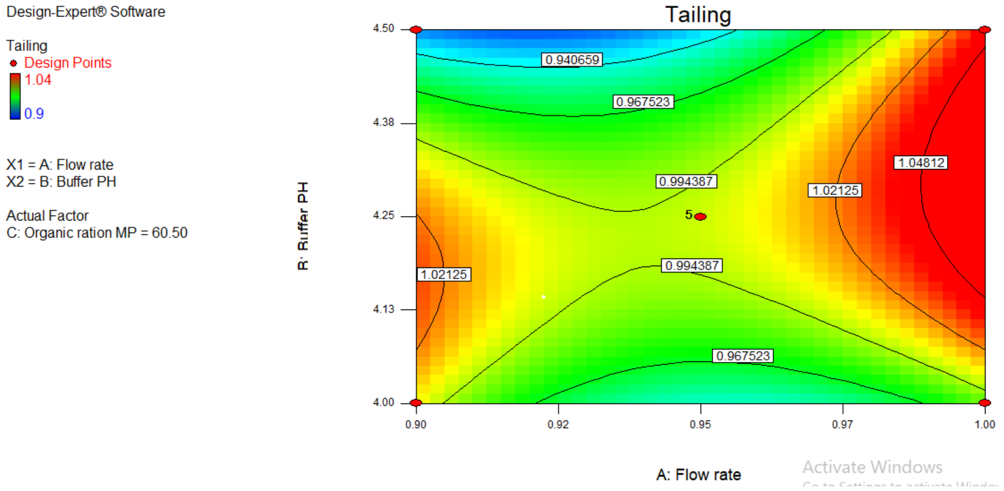

Figure 12: R tailing factor for SUB and DUB.

SYSTEM SUITABILITY

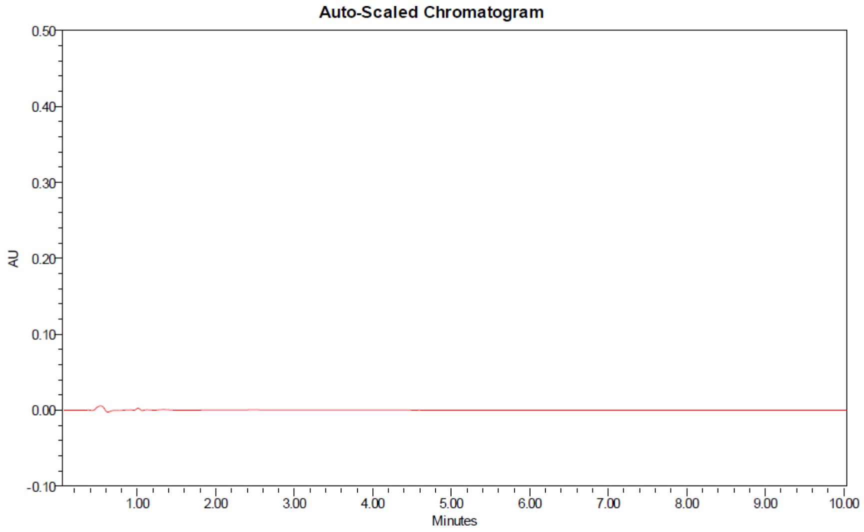

Figure 13: CG for system suitability.

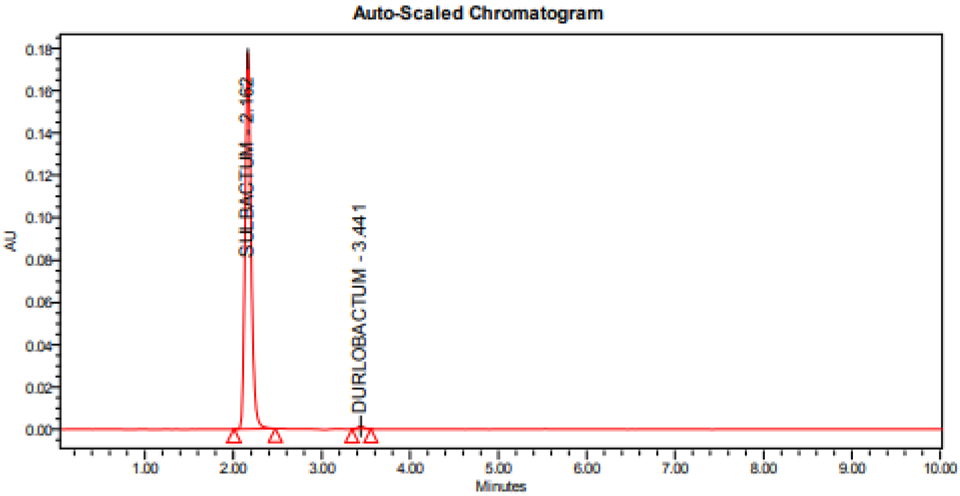

Figure 14: CG for system suitability.

Table 10: Results of system suitability parameters

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

Acceptance requirements: • There must be at least two resolutions between two medications.

VALIDATION PARAMETERS

1. ASSAY

The corresponding CGs and results are shown below.

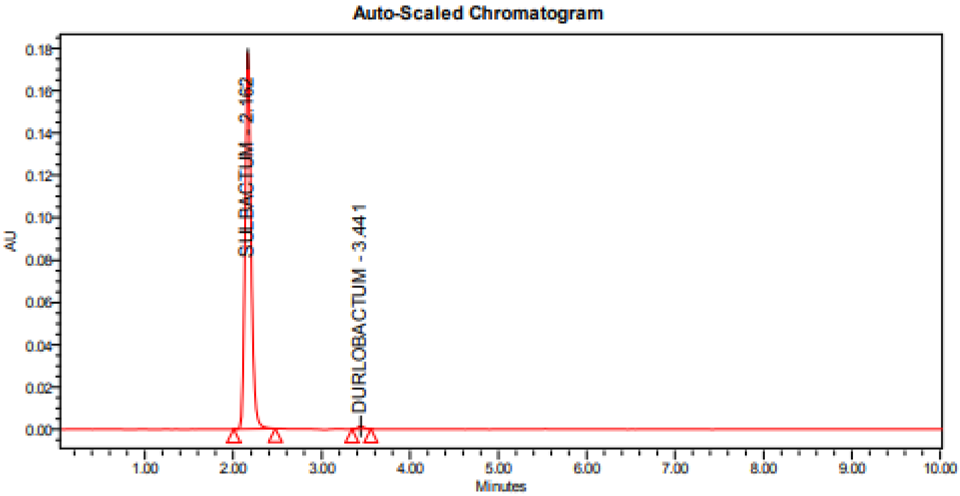

Figure 15: CG for Standard.

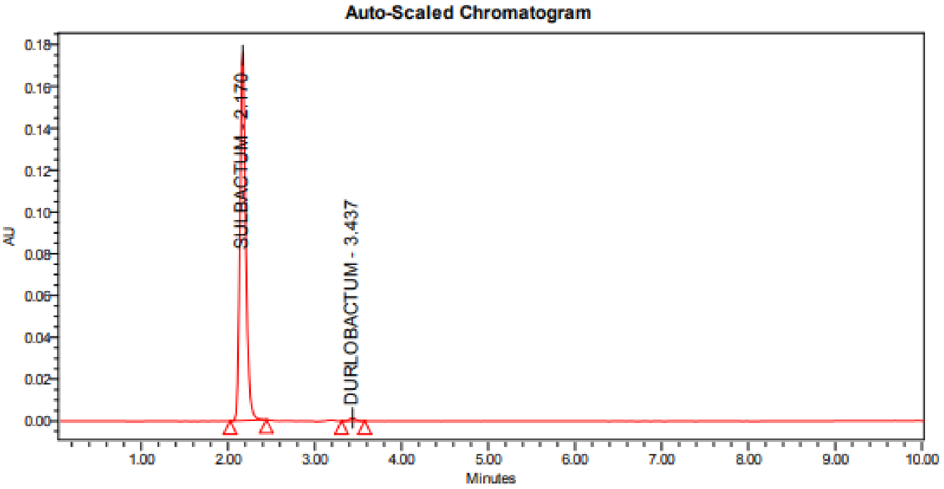

Figure 16: CG for Sample.

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

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

Table 11: Results of Assay for SUB and DUB.

	Label Claim (mg)	% Assay
SUB and DUB	0.5 g + 1.0 g	101.2

2. LINEARITY

Establish to lie from 10µg/ml to 50µg/ml of SUB, DUB & CGs are shown below

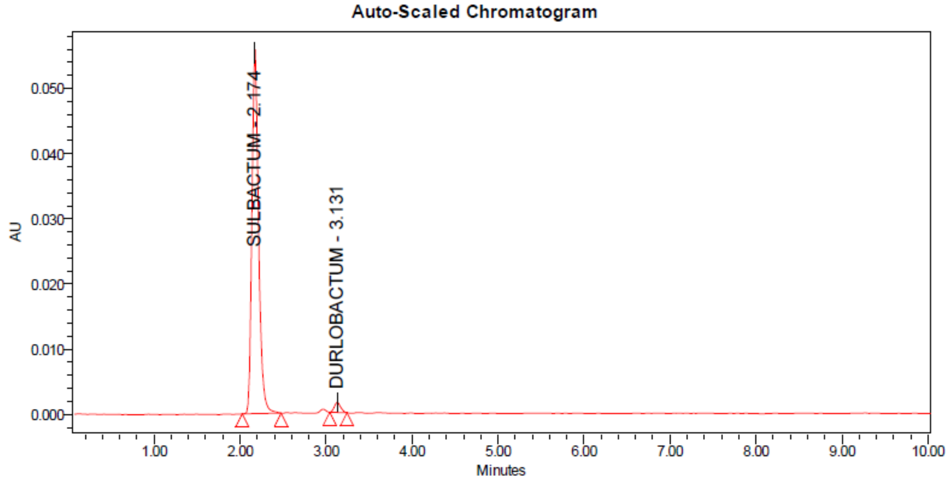

Figure 17: CG for linearity-1.

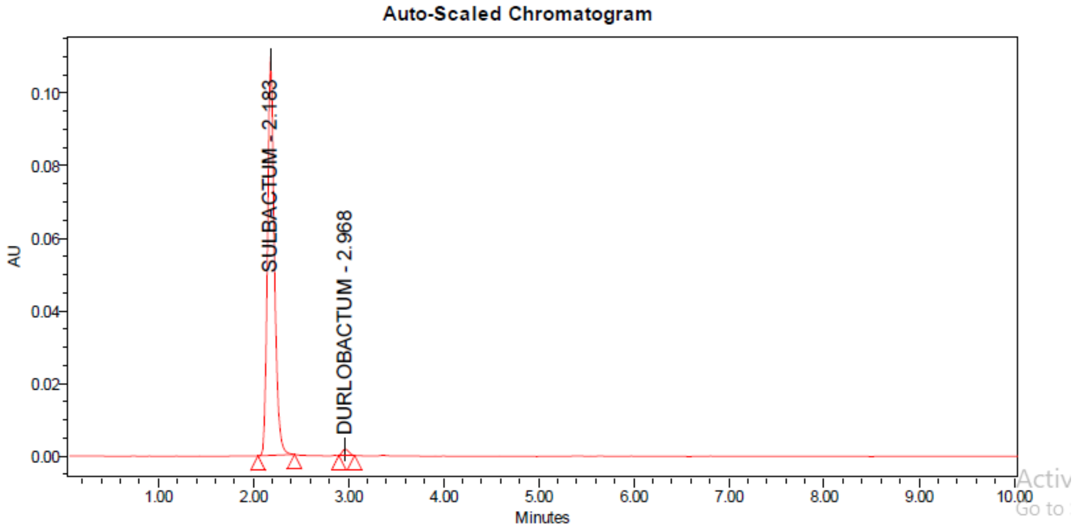

Figure 18: CG for linearity-2.

Table 12: Area of different Con.c of SUB and DUB.

S.No	Con.c (µg/ml) of SUB andDUB	Areas of DUB	Areas of SUB
1	10	6299	273312
2	20	12599	526625
3	30	18899	789938
4	40	25198	1053250
5	50	32498	1316563

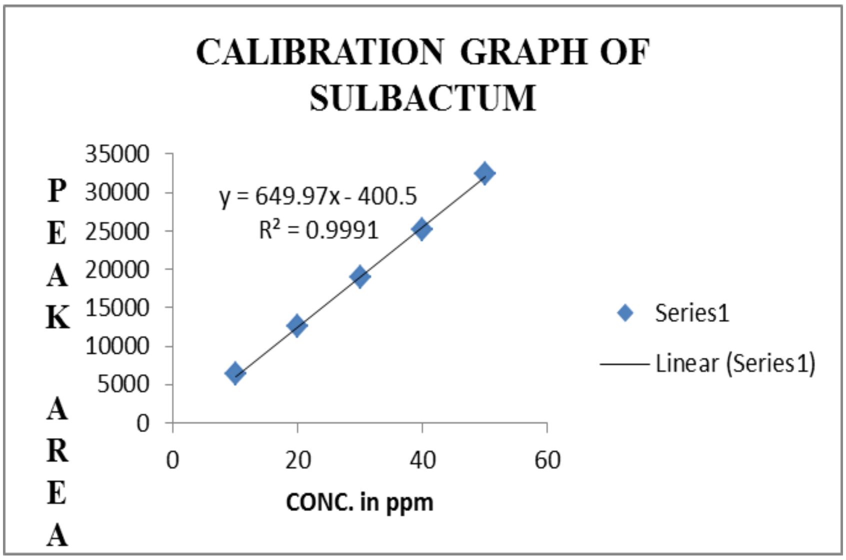

Figure 19: Calibration graph for SUB.

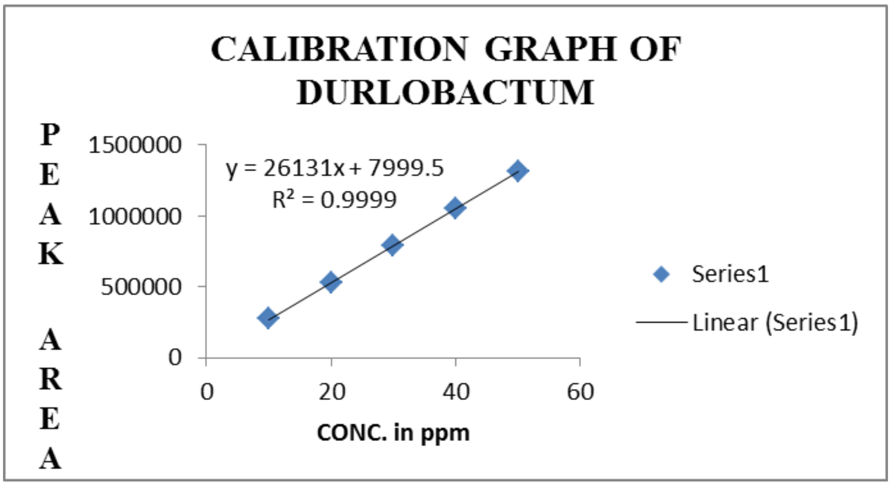

Figure 20: Calibration graph for DUB.

Table 13: Analytical performance parameters of SUB and DUB.

Parameters	SUB	DUB
Slope (m)	649.97	26131
Intercept (c)	400.5	7999.5
R^2	0.999	0.999

Acceptance criteria

The obtained R^2 of 0.999 is within the acceptable range

PRECISION

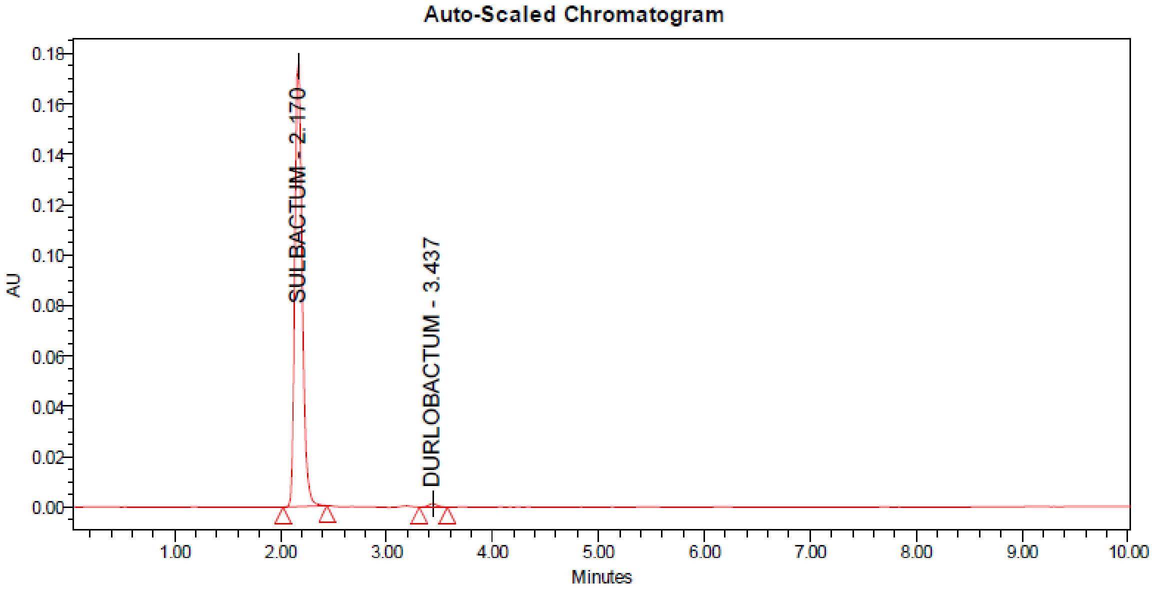

Figure 21: CG for Precision -1.

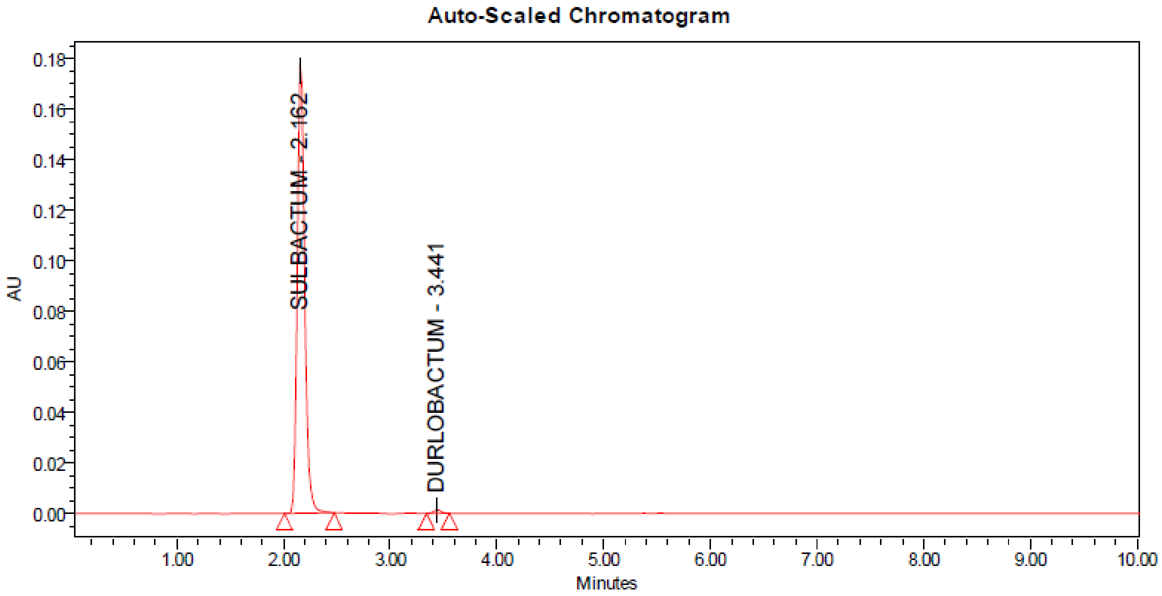

Figure 22: CG for Precision -2.

Table 14: Results of Precision for SUB and DUB.

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

- The approach is exact since the %RSD for the standard solution is below 1,
- Which is within the limitations, and the %RSD for the sample should be NMT 2.

INTERMEDIATE PRECISION (ruggedness)

At various levels of ruggedness, such as daily and system-to-system variation, there was no discernible change in the assay content or system appropriateness criteria.

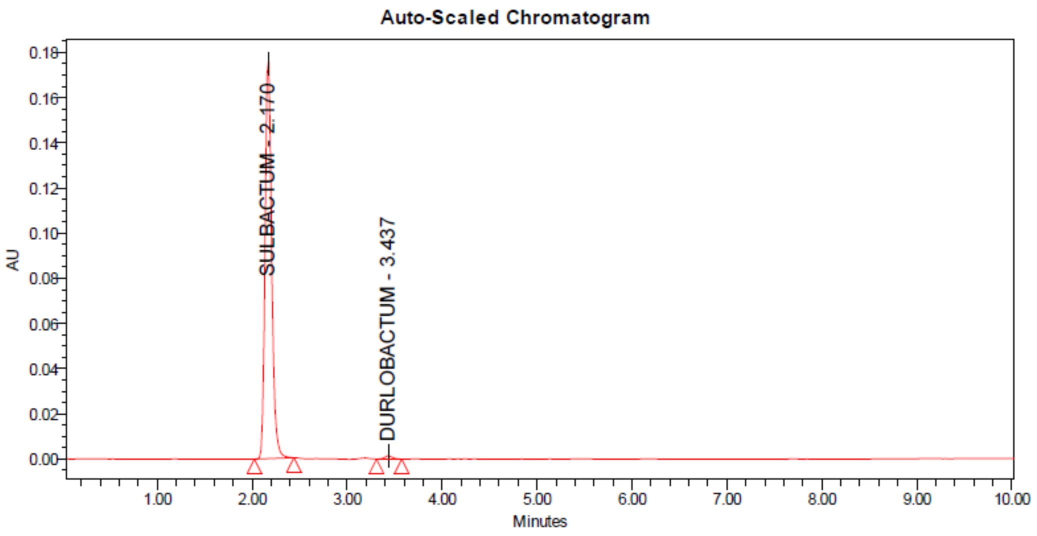

Figure 23: CG for ID Precision -1

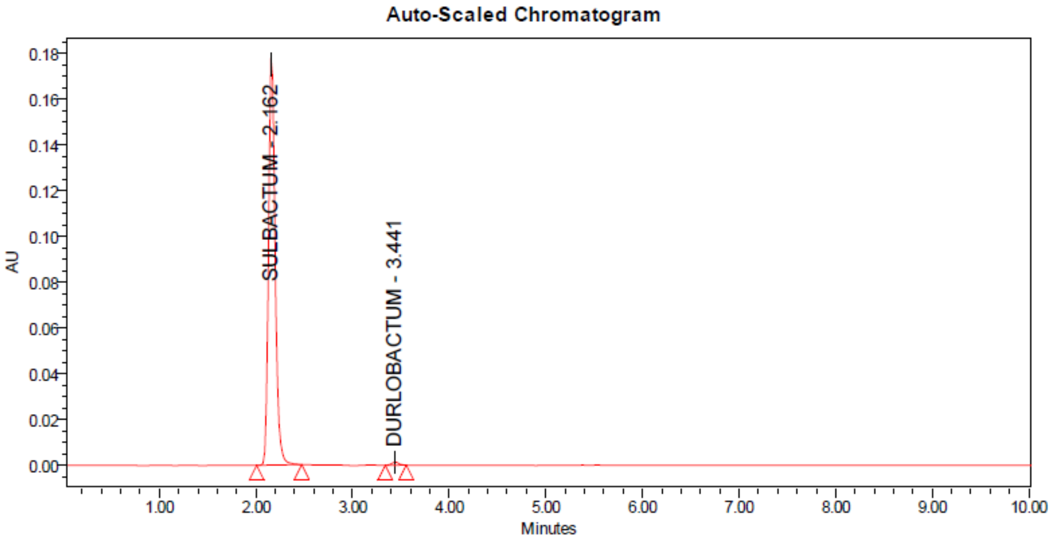

Figure 24: CG for ID Precision-2.

Table 15: Results of Intermediate precision for SUBDUB.

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

- Since the %RSD achieved is under the limit and the five distinct sample solutions' %RSD should not exceed 2, the approach is robust.

ACCURACY

Sample solutions at different Con.c s (50%, 100%, and 150%) were prepared

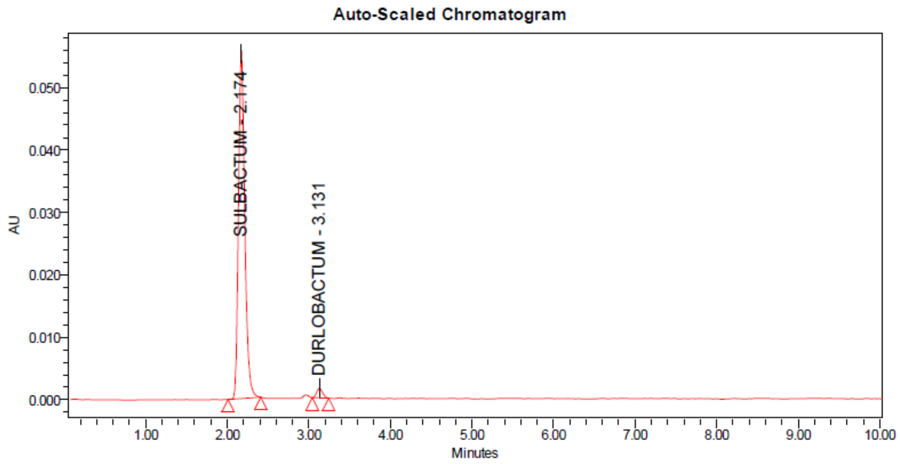

Figure 25: CG for Accuracy 50%-1.

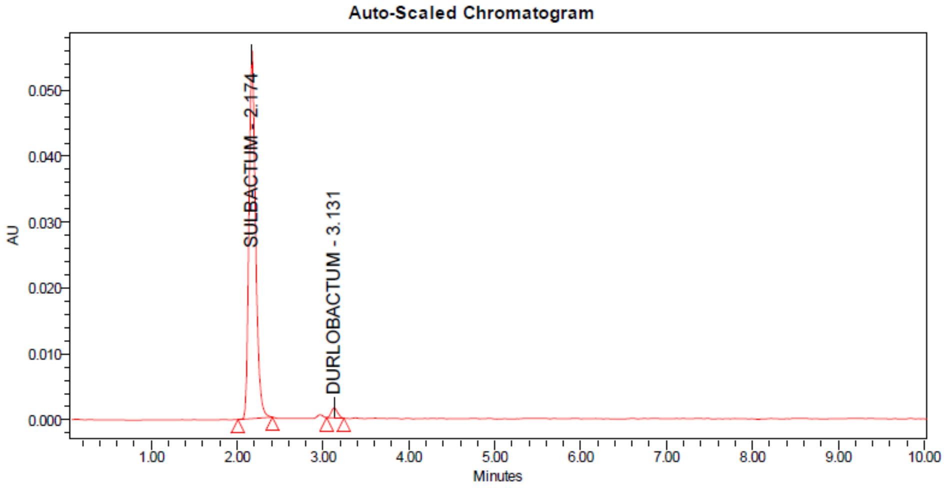

Figure 26: CG for Accuracy 50%-2.

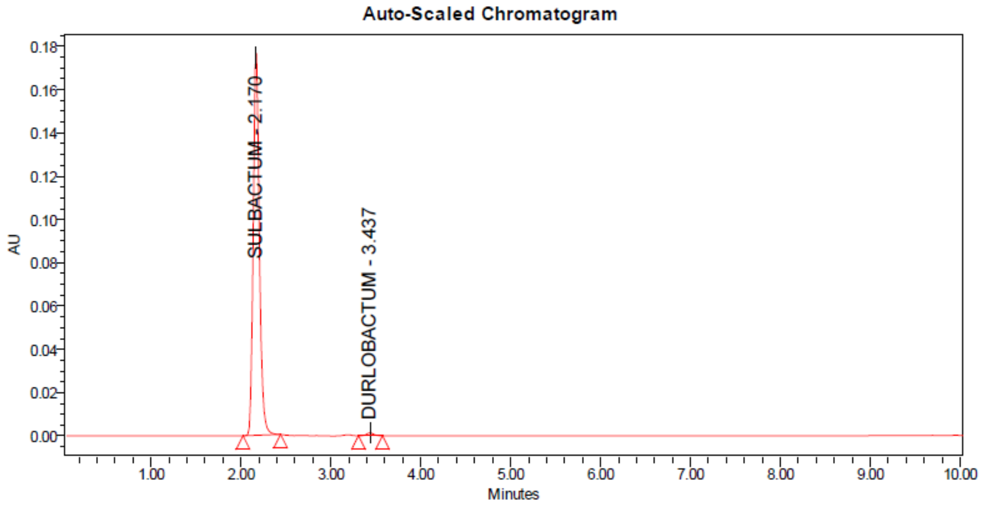

Figure 27: CG for Accuracy 100%-1.

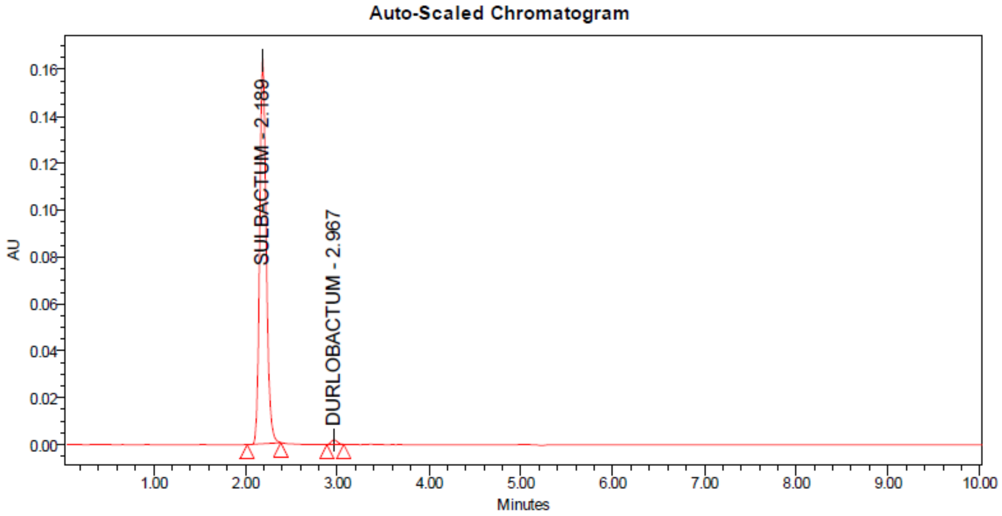

Figure 28: CG for Accuracy 100%-2.

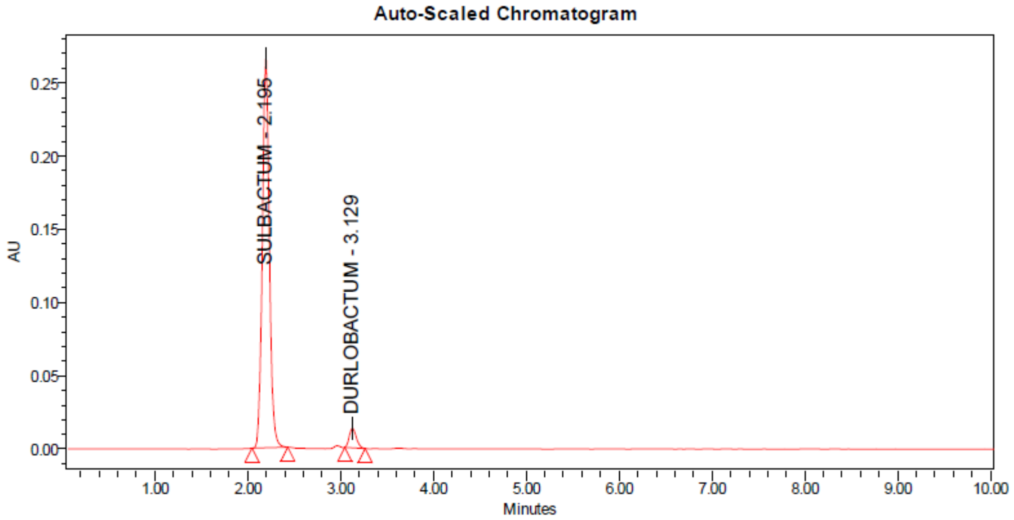

Figure 29: CG for Accuracy 150%-1.

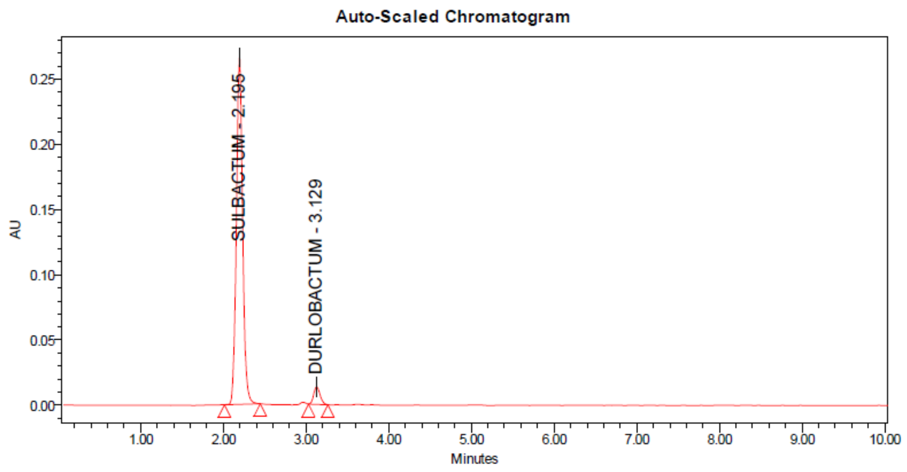

Figure 30: CG for Accuracy 150%-2.

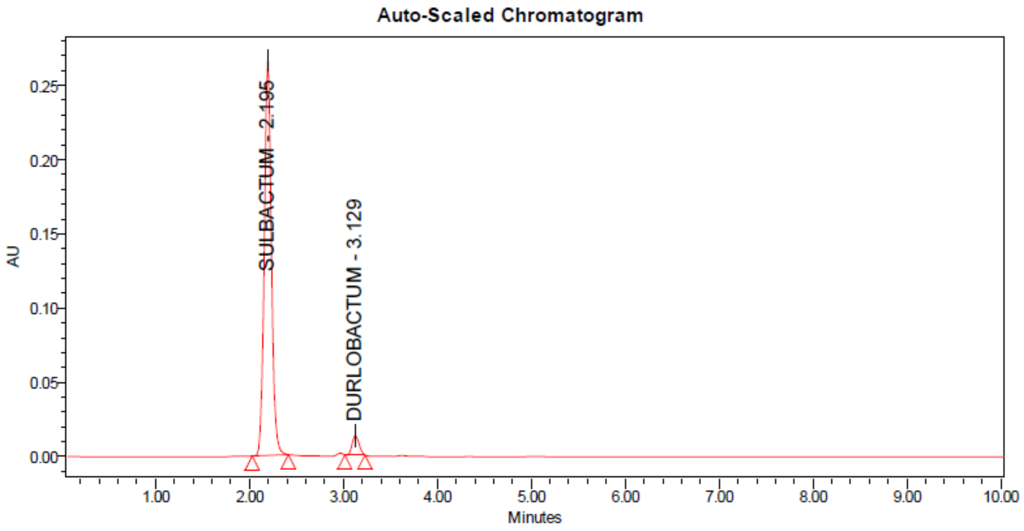

Figure 31: CG for Accuracy 150%-3.

Table 16: Accuracy (recovery) data for SUB and DUB.

%Con.c (at specification Level)	Area* of SUB	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	

%Con.c (at specification Level)	Area* DUB	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 % recovery was established to be contained by the limit (98-102%).

3. LIMIT OF DETECTION FOR SUB AND DUB

The lowest Con.c of the sample was ready with respect to the base line noise and measured the signal to noise ratio.

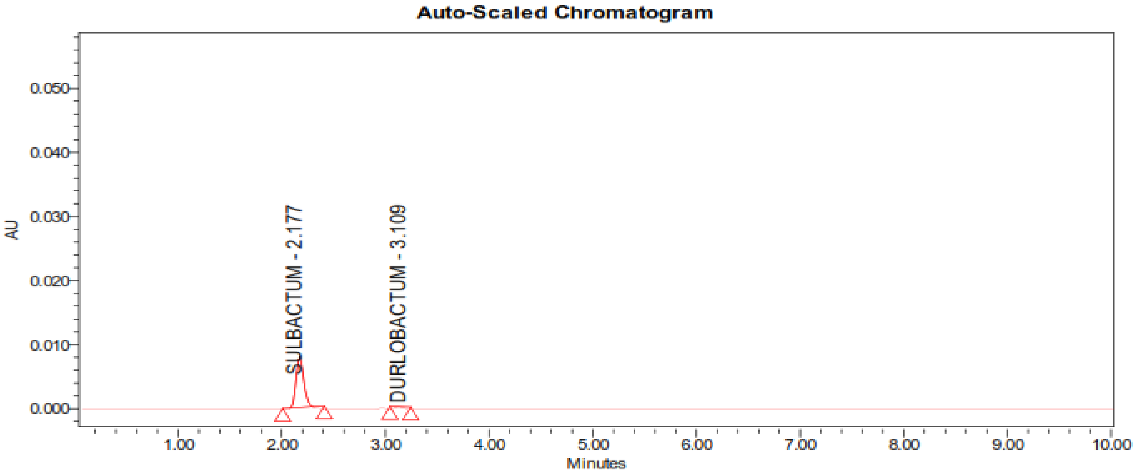

Figure 32: CG of SUB and DUBshowing LOD.

Table 17: Results of LOD.

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

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

LOQ FOR SUB AND DUB

The lowest Con.c of the sample was prepared with respect to the base line noise and Measured the signal to noise ratio.

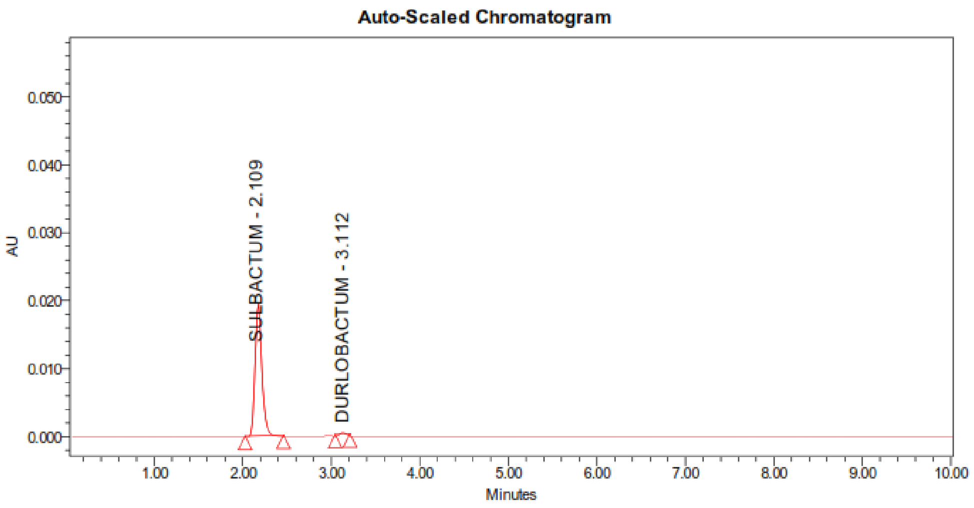

Figure 33: CG of SUB and DUBshowing LOQ.

Table 18: Results of LOQ.

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

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

4. ROBUSTNESS

The chromatographic settings were changed in order to inject the standard and the SUB and DUB samples. The resolution, tailing factor, asymmetry factor, and plate count did not vary much.

Variation in flow

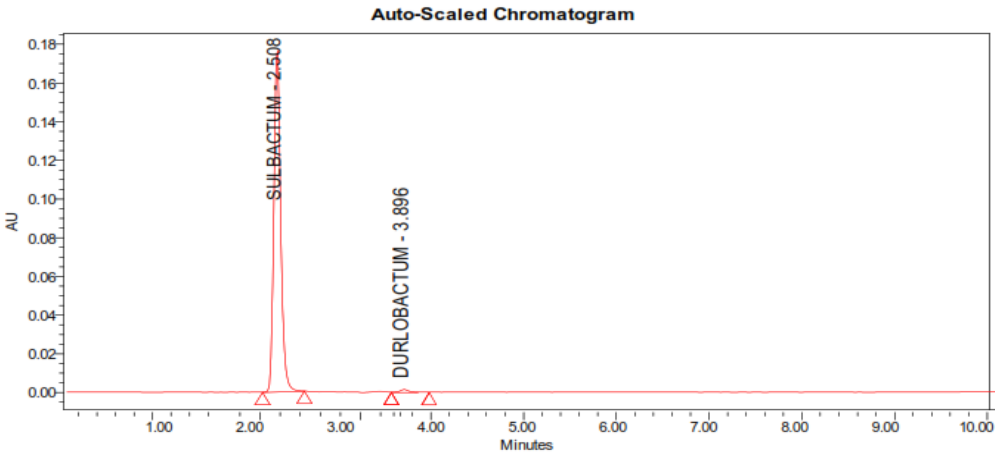

Figure 34: CG showing less flow.

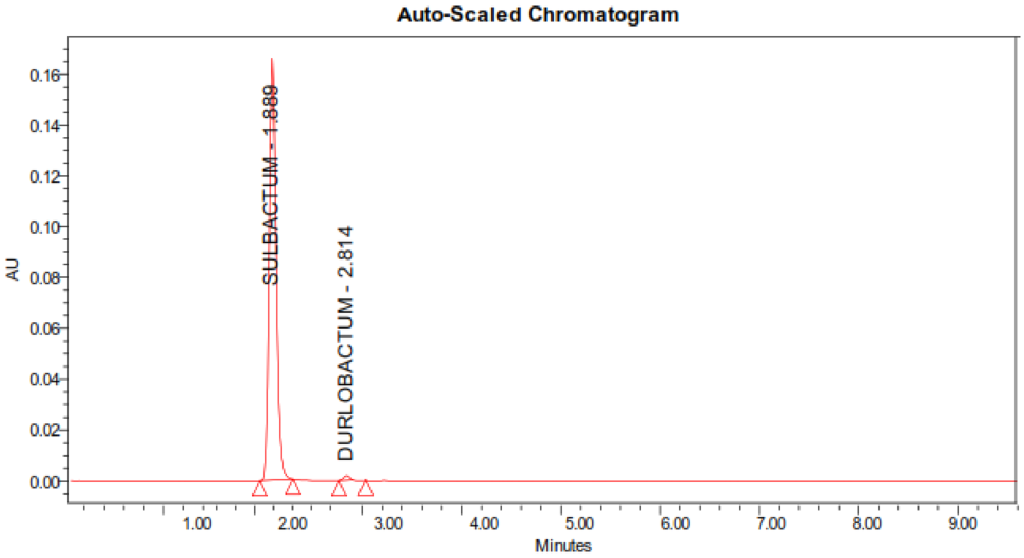

Figure 35: CG showing more flow.

Table 19: Results for variation in flow for SUB and DUB.

S. No	Flow Rate (ml/min)	System Suitability Results of SUB		System Suitability Results of DUB	
		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

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

Table 20: Results for variation in MPs composition for SUB and DUB.

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results of SUB		System Suitability Results of DUB	
		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 MPs composition have been considered from Accuracy standard.

Acceptance criteria

It was discovered that the USP tailing factor, retention duration, USP plate count, and variation in the MPs obtained for changes in flow rate were all within the acceptable range. Thus, the approach is reliable.

CONCLUSION

The reliability and efficiency of the HPLC technique for the simultaneous measurement of SUB and DUB are improved when QbD principles are applied throughout the method's development and validation. Significant improvements are made to the technique's robustness and applicability for pharmaceutical analysis by methodically identifying and manipulating important factors throughout method development.

Establishing a comprehensive process for method validation also guarantees that the procedure satisfies regulatory standards for robustness, accuracy, precision, linearity, and specificity. The validated approach adds to the overall QC and QA in pharmaceutical manufacturing processes by offering a dependable tool for routine analysis of DUB and SUB in pharmaceutical dosage forms.

In conclusion, by stressing a proactive and methodical approach to method optimization and validation, the QbD approach not only advances the scientific knowledge of method development but also encourages the manufacturing of pharmaceutical goods of the highest caliber.

REFERENCE

1. Someshwar Komati, Suryakanta Swain, Muddana Eswara Bhanoji Rao, Bikash Ranjan Jena, Sambamoorthy Unnam, Vishali Dasi, QbD-based design and characterization of mucoadhesive microspheres of quetiapine fumarate with improved oral bioavailability and brain biodistribution potential, Bulletin of Faculty of Pharmacy, Cairo University, 2018; 56(2): 129-145, ISSN 1110-0931, https://doi.org/10.1016/j.bfopcu.2018.09.002.
2. Sunil, J., Sanjith Nath, M., Samba Moorthy, U., HPLC method development and validation for simultaneous estimation of artemether and lumefantrine. Int J Pharm Pharmaceut Sci., 2010; 2: 93-96.
3. U. Sambamoorthy, Sunil J, Nath M S. HPLC Method development and validation for simultaneous estimation of ibuprofen and pseudoephedrine in pharmaceutical dosage forms. Asian Journal of Research in Chemistry, 2010; 3(4): 859-61.
4. K. Spandana, N. Venkatesh, K. Suresh, U. Sambamoorthy, A novel RP-UPLC method for the simultaneous estimation of domperidone and rabeprazole in pharmaceutical dosage form, J. Pharm, Biomed. A. Lett., 2015; 3(1): 185-192.
5. Sambamoorthy Unnam, et.al., Liposomal Melphalan: Approach to obtain improved plasma stability, pharmacokinetics, and in vitro and in vivo anticancer efficacy in

combination with liposomal simvastatin against mouse RPMI-8226 multiple myeloma model, Journal of Drug Delivery Science and Technology, 2022; 73: 103479, ISSN 1773-2247, https://doi.org/10.1016/j.jddst.2022.103479.

6. S. H. Rasheed, M. Arief, P. S. Vani, S. R. Gajavalli, G. Venkateswarlu, K. S. Hussain, N. V. Kumar, and Y. K. Reddy, “Simultaneous estimation of rabeprazole sodium and itopride hydrochloride in capsule dosage form by UV spectrophotometry,” Research Journal of Pharmacy and Technology, 2011; 4(4): 558–560.
7. Y. Krishna Reddy, *et al.* Formulation and in vitro evaluation of fesoterodine fumarate sustained release tablets Indo Am J Pharmaceut Sci., 2018; 5(3): 1448-1460, 10.5281/ZENODO.1199058
8. Y. Krishna Reddy, *et al*. Formulation and Evaluation of Extended Release Tablets of an Anti-Bacterial Drug Cephalexin, Res. J. Pharma. Dosage Forms and Tech., 2020; 12(2): 53-57. DOI: 10.5958/0975-4377.2020.00009.9
9. Sarojini et al. Formulation Development and Evaluation of Olmesartan Medoxamil Ororetentive Jellies, ejbps, 2018; 5(4): 899-906.
10. HaginakaJ, Yasuda H, Uno T, Nakagawa T. SUB: degradation and determination by high-performance liquid chromatography. Chemical and pharmaceutical bulletin, 1984; 25; 32(7): 2752-8.
11. Qi ML, Chen RL, Cong RH, Wang P. A validated method for piperacillin sodium and SUBsodium by HPLC. Journal of liquid chromatography & related technologies, 2003 Mar 1; 26(4): 665-76.
12. Dhandapani B, Thirumoorthy N, Rasheed SH, Kotaiah MR, Chandrasekhar K. RP-HPLC method development and validation-cefoperazone and SUB. Int J Chem Tech Res., 2010; 3: 752-5.
13. Shrestha B, Bhuyan NR, Sinha BN. HPLC method for estimation of ceftriaxone and SUB. International Journal of PharmTech Research, 2012 Oct; 4(4): 1660-6.