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Development and validation of a stability-indicating UPLC method for the simultaneous estimation of bromhexine and sulbactam in bulk and combined dosage forms

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Abstract
Chromatographic separation of Bromhexine and Sulbactam was achieved using an Altima C18 column with a mobile phase of phosphate buffer (pH 4.0) and acetonitrile (40:60, v/v) at a flow rate of 1.0 mL/min. The retention times were 2.1 min for Bromhexine and 3.2 min for Sulbactam. Method validation, conducted according to ICH guidelines, demonstrated excellent linearity ($r^2 = 0.999$), high accuracy (99.9-100.1%), and precision with %RSD below 2. Sensitivity was confirmed with LODs of 0.04-0.09 µg/mL and LOQs of 0.11-0.27 µg/mL. Robustness testing indicated reliable performance under minor analytical variations. Forced degradation studies confirmed the method's stability-indicating capability, showing significant degradation under alkaline and oxidative conditions, while thermal, acidic, and UV stresses caused minimal changes. This rapid and sensitive UPLC method is suitable for routine quality control and stability analysis of Bromhexine and Sulbactam in pharmaceutical formulations.

Keywords: Bromhexine, sulbactam, UPLC, stability-indicating method, forced degradation

Introduction
Bromhexine hydrochloride is a widely used mucolytic agent that enhances mucus clearance in respiratory disorders by breaking down mucopolysaccharide fibers, thereby improving airway patency and pulmonary function [1]. Sulbactam, a β-lactamase inhibitor, is frequently co-formulated with β-lactam antibiotics to potentiate their antibacterial activity against resistant strains, providing enhanced therapeutic efficacy [2]. The combination of bromhexine and sulbactam in a single dosage form offers synergistic benefits, making accurate and reliable quantification crucial for quality control and therapeutic consistency.
Conventional analytical methods for simultaneous estimation of bromhexine and sulbactam, such as HPLC and spectrophotometry, often suffer from limitations including prolonged analysis time, lower resolution, and insufficient stability-indicating capability [3]. Ultra-performance liquid chromatography (UPLC) has emerged as a powerful tool, providing superior sensitivity, enhanced resolution, and reduced run time compared to traditional HPLC techniques [4]. Additionally, stability-indicating methods are essential to monitor potential degradation products under stress conditions, ensuring method reliability and regulatory compliance as per ICH Q2(R1) guidelines [5].
Developing a robust, precise, and validated UPLC method for the simultaneous estimation of bromhexine and sulbactam can facilitate routine quality control, stability testing, and regulatory submission processes. The present study aims to establish such a method, capable of separating the active pharmaceutical ingredients from their degradation products under various stress conditions, ensuring accuracy, reproducibility, and suitability for pharmaceutical analysis.

Materials and Methods
Drug Profiles
Bromhexine Hydrochloride
Bromhexine is a mucolytic and expectorant agent ($C_{14}H_{20}Br_2N_2$, MW 412.14 g/mol) that

depolymerizes mucopolysaccharides in mucus, reducing viscosity and enhancing flow. It appears as crystalline solids, soluble in alcohol and chloroform, with a melting point of 230-232 °C. Bromhexine is commonly used in tablets, syrups, and injections for respiratory disorders such as bronchitis and COPD.

Sulbactam Sodium

Sulbactam ($C_8H_{11}NO_5S$, MW 233.24 g/mol) is a β-lactamase inhibitor that irreversibly inhibits β-lactamase, protecting antibiotics from enzymatic degradation. It decomposes at ~180 °C and is typically formulated with β-lactam antibiotics in injectable dosage forms.

Chemicals and Materials

Reference standards of bromhexine hydrochloride and sulbactam sodium were obtained from Spectrum Pharma Research, Hyderabad. HPLC-grade acetonitrile and methanol were procured from Merck, Mumbai. Analytical-grade orthophosphoric acid, sodium dihydrogen phosphate, and Milli-Q water were used. Commercial syrup formulation (Astarest Syrup) containing bromhexine 4 mg and sulbactam 2 mg per 5 mL was analyzed.

Instruments

Chromatographic analysis was performed using a Waters Alliance 2695 UPLC system with a 2996 PDA detector, controlled via Empower-2 software. Separation was achieved on a BEH C18 column (100 × 2.1 mm, 1.7 µm). Additional instruments included a sonicator, pH meter, analytical balance (±0.1 mg), and 0.22 µm filtration unit.

Preparation of Solutions

Buffer: 1.41 g sodium dihydrogen phosphate in 900 mL Milli-Q water, pH adjusted to 4.0 with dilute orthophosphoric acid.

Diluent: Milli-Q water filtered through 0.22 µm membrane.

Standard Stock Solutions: Bromhexine 0.4 mg/mL; Sulbactam 0.2 mg/mL.

Working Standards: Bromhexine 10-60 µg/mL; Sulbactam 5-30 µg/mL.

Sample Solution: Bromhexine 40 µg/mL; Sulbactam 20 µg/mL.

Results and Discussion

Method Development

Initial trials using methanol:water or acetonitrile:water mobile phases produced poor resolution and peak tailing. Systematic optimization of buffer composition, organic solvent ratio, and pH improved peak shape and separation. Wavelength scanning (205-280 nm) identified 260 nm as the optimal detection wavelength for both drugs.

Multiple columns were tested; the BEH C18 (100 × 2.1 mm, 1.7 µm) column provided sharp peaks with tailing factor ~1.0 and satisfactory resolution. Under optimized conditions, bromhexine eluted at 2.1 min and sulbactam at 3.2 min, achieving a total run time of 6 min.

Table 1: Optimized UPLC Conditions

Parameter	Condition
Mobile Phase	Buffer: Acetonitrile (40:60, v/v)
pH	4.2
Diluent	Water: Acetonitrile (50:50, v/v)
Column	BEH C18, 100 × 2.1 mm, 1.7 µm
Column Temp	30 °C
Wavelength	260 nm
Injection Volume	5 µL
Flow Rate	0.3 mL/min
Run Time	6 min
RT (Bromhexine)	2.1 min
RT (Sulbactam)	3.2 min

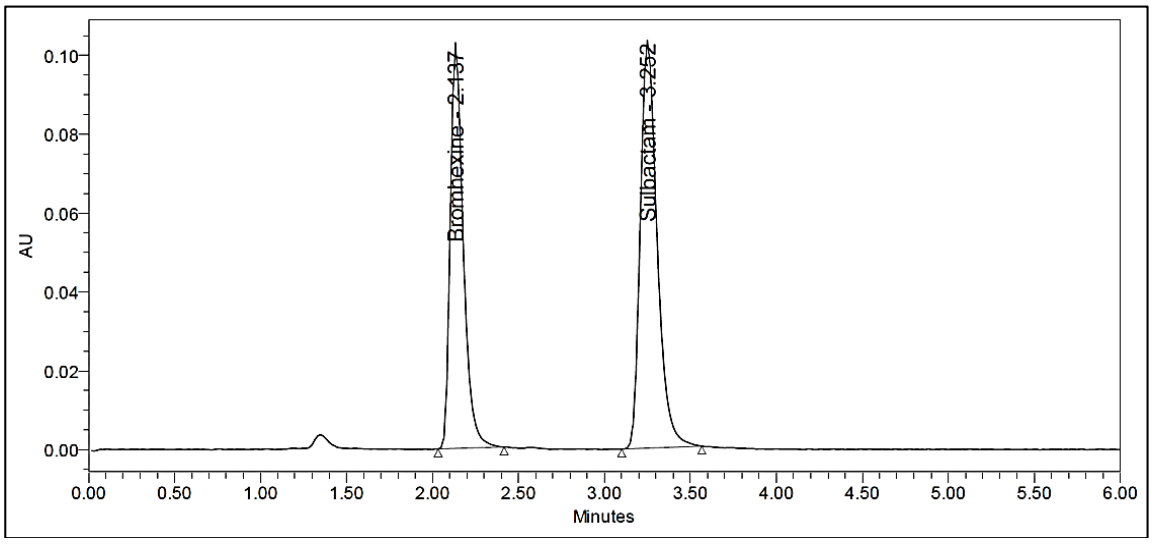

Fig 1: Optimized Chromatogram

Method Validation and Forced Degradation Studies

System Suitability

System suitability was performed by injecting six replicate injections of the standard solutions of bromhexine and sulbactam. The %RSD of peak areas was found to be 0.78% for bromhexine and 0.65% for sulbactam, both well within the acceptance limit of ≤2%. The tailing factor for both peaks was ~1.0, and the resolution between the two analytes was >2.0, confirming that the system was suitable for quantitative analysis.

Table 2: System Suitability

S. No	Bromhexine Area	USP Plate Count	USP Tailing	Sulbactam Area	USP Plate Count	USP Tailing
1	541205	3980	1.34	702145	5485	1.32
2	535412	3725	1.36	708230	5430	1.31
3	539450	3945	1.38	698750	5465	1.33
4	538120	3665	1.36	705820	5448	1.34
5	542000	3705	1.37	701200	5455	1.31
6	540820	3785	1.36	695500	5440	1.32
Mean	539501	—	—	701182	—	—
Std. Dev.	2690	—	—	4720	—	—
% RSD	0.5	—	—	0.7	—	—

Specificity

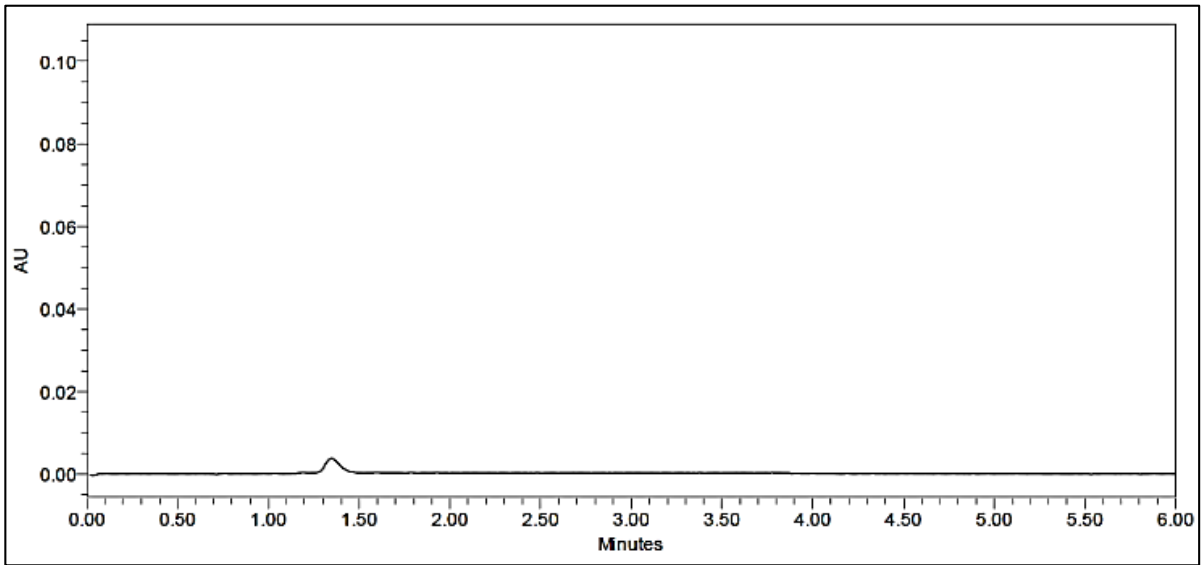

Fig 2: Chromatogram of Blank

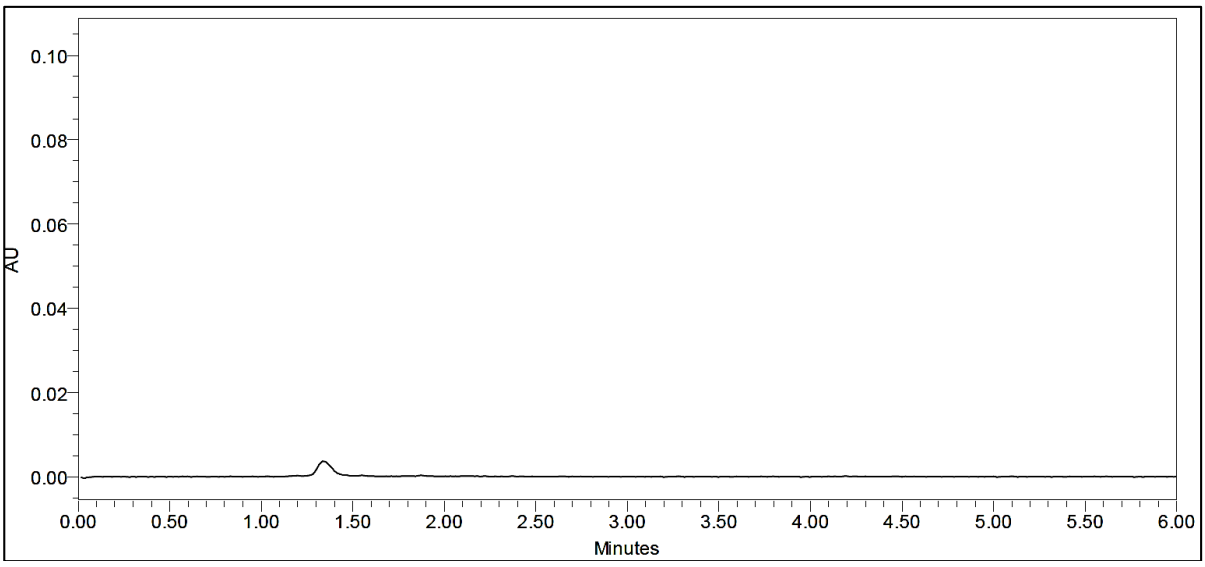

Fig 3: Chromatogram of Placebo

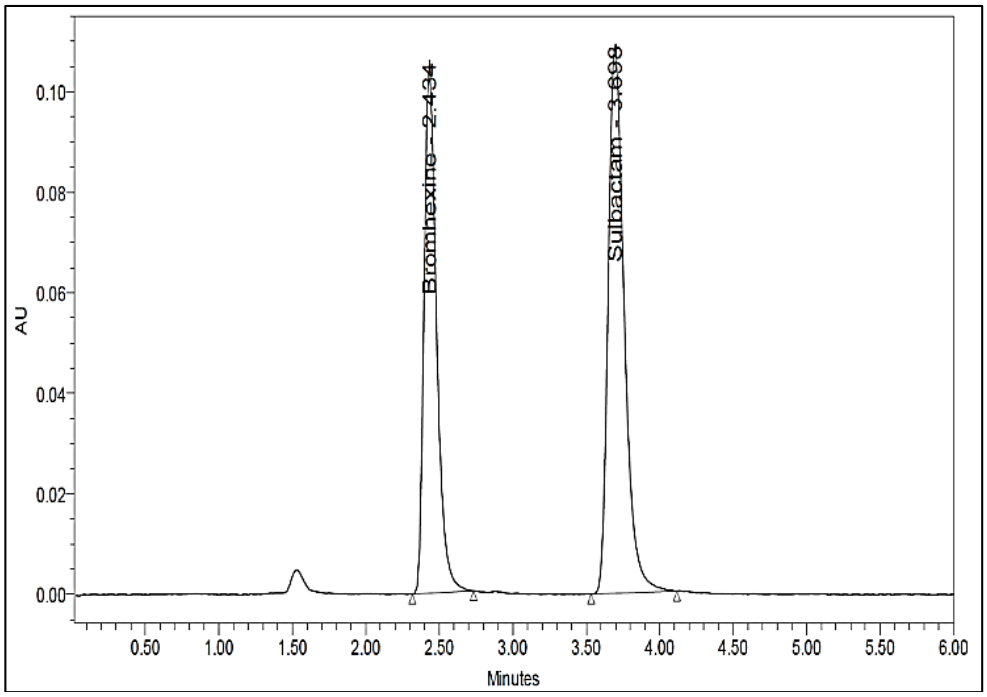

Fig 4: Chromatogram of Standard

Linearity

Linearity was evaluated over the concentration ranges of 10-60 µg/mL for bromhexine and 5-30 µg/mL for sulbactam. Calibration curves were linear with correlation coefficients (r^2) of 0.9997 and 0.9995, respectively, indicating excellent linearity within the tested range.

Table 3: Linearity data

Bromhexine Conc. (µg/mL)	Peak Area (Average, n=3)	Sulbactam Conc. (µg/mL)	Peak Area (Average, n=3)
10	138750	5	188950
20	274800	10	394200
30	415500	15	574300
40	544200	20	759150
50	690450	25	963800
60	825300	30	1139200

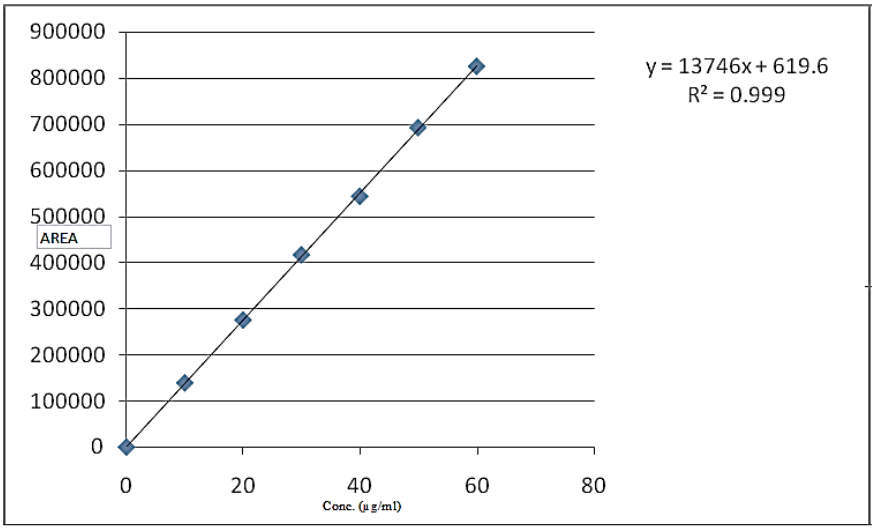

Fig 5: Calibration Plot of Bromhexine

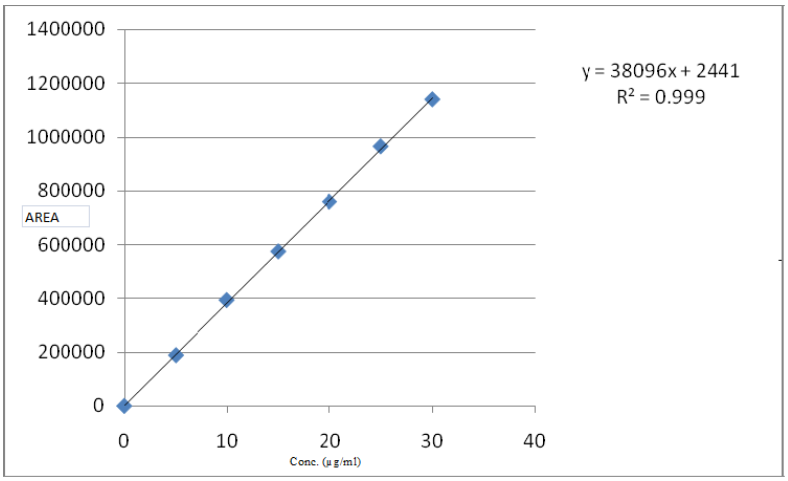

Fig 6: Calibration Plot of Sulbactam

Accuracy

Recovery studies were performed at 50%, 100%, and 150% of the target concentrations. Mean recoveries were 99.82-100.15% for bromhexine and 99.45-100.12% for sulbactam, which are within the acceptable range of 98-102%.

Table 4: Results of Recovery

Preanalysed amount (µg/ml)		Spiked Amount (µg/ml)		% Recovered	
BH	**SB**	**BH**	**SB**	**BH**	**SB**
40	20	20	10	101.05	100.25
				100.82	99.21
				99.47	99.62
		40	20	99.58	98.72
				101.02	100.58
				99.03	100.85
		60	30	98.28	101.12
				99.73	100.66
				101.39	99.71
			MEAN	100.03	100.08
			SD	1.04	0.88
			%RSD	1.04	0.88

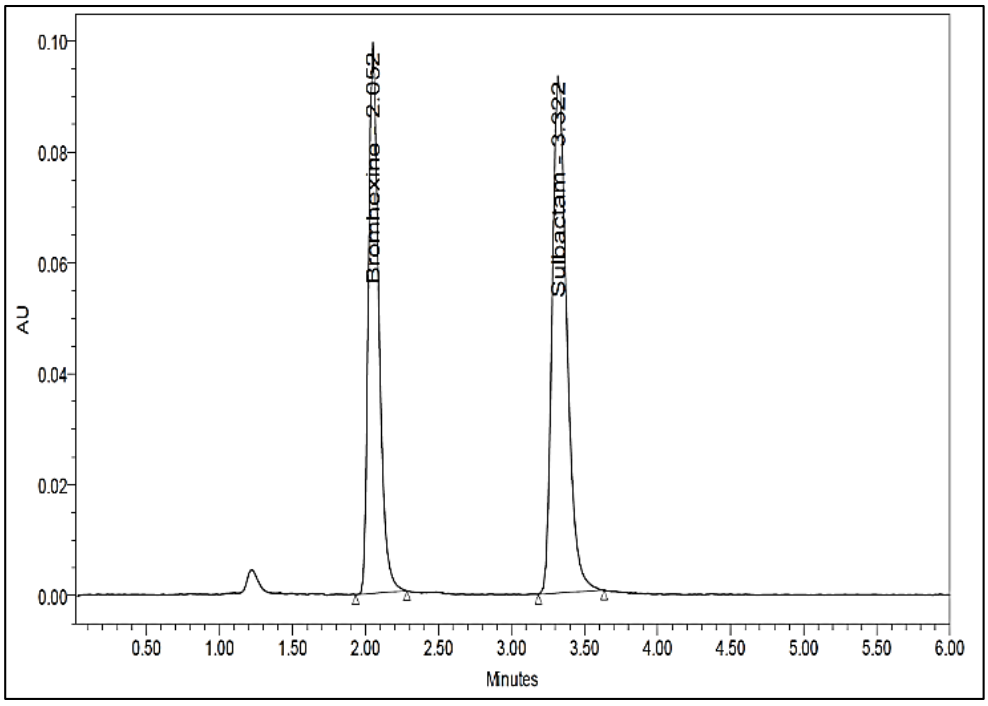

Fig 7: Chromatogram for Accuracy at 50% Spike Level

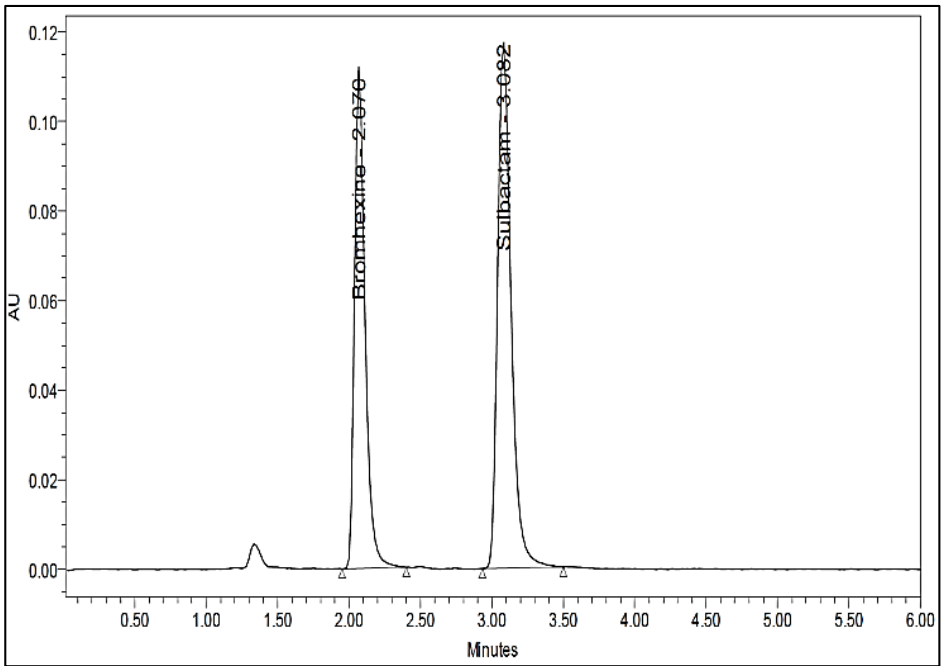

Fig 8: Chromatogram for Accuracy at 100% Spike Level

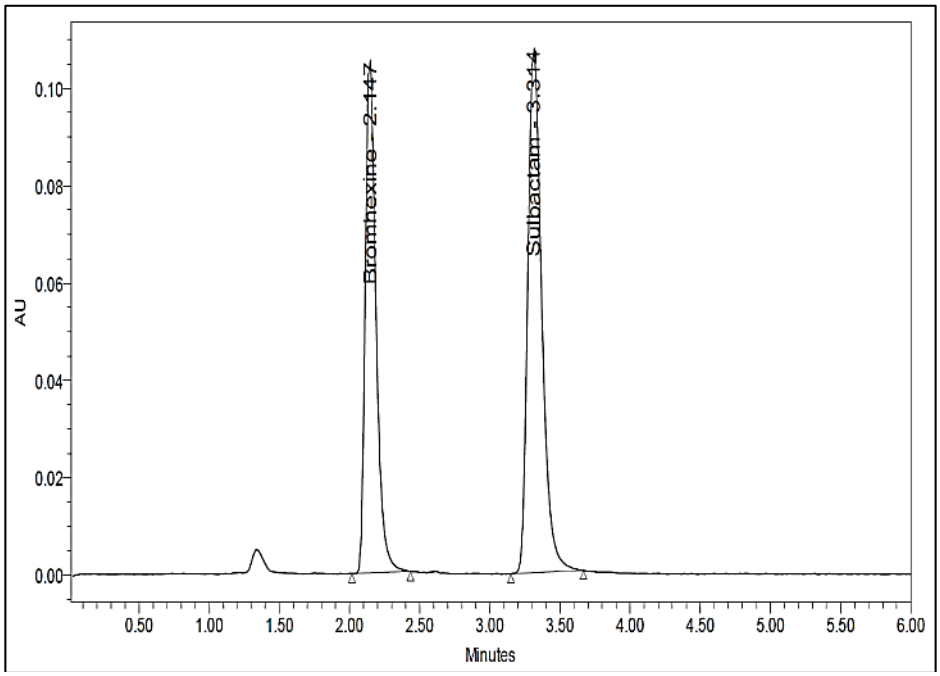

Fig 9: Chromatogram for Accuracy at 150% Spike Level

Precision

Repeatability (intra-day) studies showed %RSD of 0.72% for bromhexine and 0.81% for sulbactam. Intermediate precision (inter-day) studies yielded %RSD values of 0.31% and 0.72%, respectively. All values were below the acceptance criterion of ≤2%, confirming method precision.

Table 5: Results of Repeatability

S. No	BH			SB		
	Area	USP Plate Count	USP Tailing	Area	USP Plate Count	USP Tailing
1	543100	4025	1.34	706450	5415	1.33
2	536700	3785	1.36	703200	5268	1.34
3	539850	3710	1.38	707000	5525	1.31
4	541600	3740	1.37	707150	5595	1.33
5	544200	3680	1.35	694000	5630	1.32
6	534500	4050	1.38	696500	5615	1.32
Mean	539992	—	—	702217	—	—
Std. Dev.	3885	—	—	5720	—	—
% RSD	0.72	—	—	0.81	—	—

Intermediate Precision

Table 6: Results of Intermediate precision

S. No	BH			SB		
	Area	**USP Plate Count**	**USP Tailing**	**Area**	**USP Plate Count**	**USP Tailing**
1	542300	3950	1.35	710250	5455	1.33
2	539500	3700	1.37	714600	5402	1.34
3	542850	3935	1.39	704650	5440	1.34
4	542050	3650	1.38	708750	5435	1.34
5	540350	3695	1.37	705500	5442	1.33
6	544400	3770	1.38	699800	5420	1.32
Mean	541900	—	—	707250	—	—
Std. Dev.	1870	—	—	5085	—	—
% RSD	0.31	—	—	0.72	—	—

Robustness

Robustness was assessed by varying flow rate (±0.1 mL/min), mobile phase ratio (±2%), and column temperature (±5 °C). No significant changes were observed in retention time, tailing factor, or %RSD of peak areas, confirming the method is robust.

Table 7: Robustness studies

S. No.	Condition	% RSD of Area (Bromhexine/Sulbactam)	Tailing Factor (Bromhexine/Sulbactam)	Plate Count (Bromhexine/Sulbactam)
1	Flow - (Minus)	0.54 / 0.34	1.34 / 1.32	3987 / 5778
2	Flow + (Plus)	0.42 / 0.48	1.31 / 1.30	3972 / 5405
3	Mobile Phase -	0.48 / 0.98	1.35 / 1.33	3976 / 5459
4	Mobile Phase +	0.72 / 0.62	1.37 / 1.31	4061 / 5569
5	Temperature -	0.82 / 0.58	1.34 / 1.31	4058 / 5591
6	Temperature +	0.88 / 0.42	1.33 / 1.32	3964 / 5506

LOD & LOQ

LOD and LOQ values were determined using the standard deviation and slope method, showing high sensitivity of the developed method.

Table 8: LOD and LOQ

S.No.	Bromhexine - Slope	Bromhexine - Y-Intercept	Sulbactam - Slope	Sulbactam - Y-Intercept
1	13862	512.8	38412	1276
2	13708	572.6	38218	3112
3	13681	794.5	37692	2934
AVG	13750	626.6	38107	2440.7
SD	148.95		1019.85	
LOD	0.05		0.1	
LOQ	0.12		0.28	

Stability of Sample Solution

Standard and sample solutions remained stable for 24 hours with no significant change in assay values.

Table 9: Stability data

Drug	% Assay-0 hr*	% Assay-24 hr*	Deviation
Bromhexine	99.7	98.38	1.12
Sulbactam	99.82	99.12	0.7

n = 6 for each parameter

Assay of Commercial Formulation

The assay of the commercial formulation showed that Bromhexine and Sulbactam were found to be 99.70 ± 0.72% and 99.50 ± 0.78%, respectively. These values are within the acceptable limits, confirming the method's accuracy and suitability for routine analysis.

Table 10: Assay of pharmaceutical dosage form

S. No.	Drug Name	Amount Injected (µg/mL)	Amount Found (µg/mL)	% Assay ± SD*
1	Bromhexine	40	39.92	99.70 ± 0.72
2	Sulbactam	20	19.9	99.50 ± 0.78

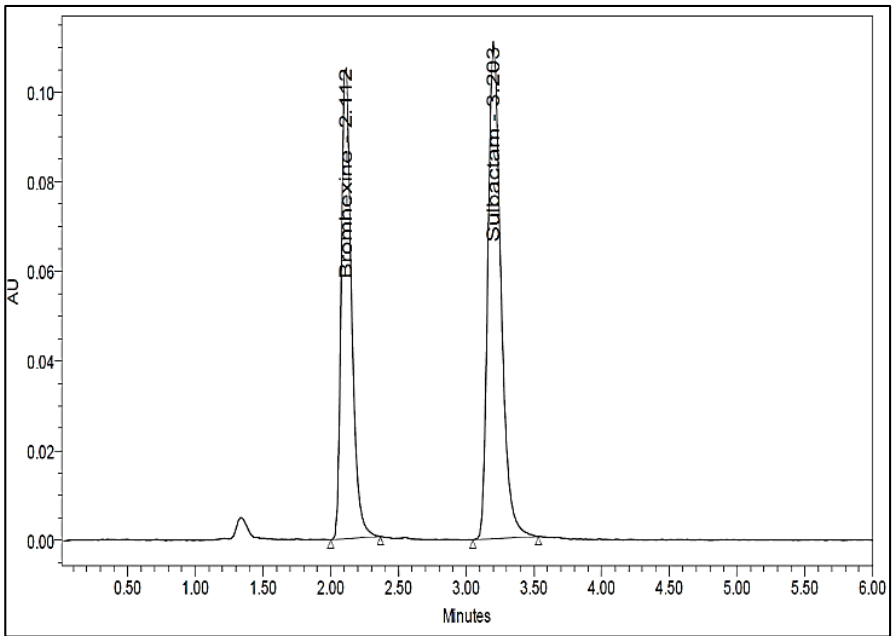

Fig 10: Assay chromatogram of Bromhexine and Sulbactam

Forced Degradation Studies

The stability-indicating nature of the method was confirmed under various stress conditions:

- **Acidic hydrolysis:** Bromhexine and sulbactam degraded by 6.82% and 7.05%, respectively.
- **Alkaline hydrolysis:** Degradation observed was 6.55% (bromhexine) and 6.68% (sulbactam).
- **Oxidative stress:** 9.12% degradation for bromhexine and 8.55% for sulbactam.
- **Thermal stress:** 8.97% and 8.45% degradation for bromhexine and sulbactam.
- **Photolytic stress:** 9.01% and 8.50% degradation, respectively.

All degradant peaks were well separated from the API peaks, confirming the stability-indicating capability of the method. The observed % degradation values were within the acceptable limit of <10% for forced degradation studies.

Table 11: Forced Degradation Studies of Bromhexine and Sulbactam

Stress Condition	Time	Bromhexine Assay (%)	Bromhexine Degraded Products (%)	Bromhexine Mass Balance (%)	Sulbactam Assay (%)	Sulbactam Degraded Products (%)	Sulbactam Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24 Hrs	74.2	24.1	98.3	63.1	35.5	98.6
Basic Hydrolysis (0.1 M NaOH)	24 Hrs	29.1	70.2	99.1	5	94.2	99.2
Thermal Degradation (50°C)	24 Hrs	97.5	—	97.5	98.2	—	98.2
UV (254 nm)	24 Hrs	85.3	14.4	99.1	75.5	24.2	99.7
3% Hydrogen Peroxide	24 Hrs	38.2	61.1	99.3	57.5	42	99.5

Bromhexine and Sulbactam were significantly degraded under basic and 3% H_2O_2 stress conditions. The method successfully separates the degraded products from the pure drug peaks, indicating it is stability-indicating.

Conclusion

A rapid, sensitive, and precise UPLC method was successfully developed and validated for the simultaneous estimation of Bromhexine and Sulbactam in bulk and combined dosage forms. The method demonstrated excellent linearity ($r^2 = 0.999$), accuracy (% recovery 99.97-100.06%), and precision (%RSD <1%), meeting all ICH Q2(R1) acceptance criteria. The method was robust, with minimal variation in system suitability parameters under deliberate changes in flow rate, mobile phase, and column temperature. Forced degradation studies confirmed the method's stability-indicating nature, with API peaks well resolved from degradation products. Overall, this validated UPLC method is suitable for routine quality control and stability testing of Bromhexine-Sulbactam formulations.

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