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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS DETERMINATION OF BROMHEXINE AND SULBACTAM IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Bromhexine is a mucolytic agent used in the treatment of respiratory disorders associated with viscid or excessive mucus. In addition, Bromhexine has antioxidant properties. Bromhexine is intended to support the body's mechanisms for clearing mucus from the respiratory tract. The present study is to develop and validate a suitable RP-HPLC method for simultaneous estimation of Bromhexine and Sulbactam in pharmaceutical dosage forms in accordance with the ICH guidelines. Altima C18 (150 x 4.6 mm, 5µm) column was selected as the stationary phase. Phosphate buffer (pH 4), acetonitrile were taken in the ratio 40:60%v/v and used as mobile phase at a flow rate of 1.0 ml/min. The retention times of Bromhexine and Sulbactam were found to be 2.1 min & 3.2 min, respectively. The study clearly shows that the developed method can be successfully employed for routine quality control of Bromhexine and Sulbactam in drug testing laboratories and pharmaceutical industries.

INTRODUCTION

Bromhexine is a mucolytic agent used in the treatment of respiratory disorders associated with viscid or excessive mucus. In addition, Bromhexine has antioxidant properties. Bromhexine is intended to support the body's mechanisms for clearing mucus from the respiratory tract. It is secretolytic, increasing the production of serous mucus in the respiratory tract and makes the phlegm thinner and less viscous. This contributes to a secretomotoric effect by helping the cilia transport the phlegm out of the lungs. For this reason it is often added to cough syrups. Bromhexine is a synthetic derivative of the herbal active ingredient Vasicine. It has been shown to increase the proportion of serous bronchial secretion, making it more easily expectorated. It is indicated as "secretolytic therapy in bronchopulmonary diseases associated with abnormal mucus secretion and impaired mucus transport". Bromhexine is contained in various formulations, high and low strength syrups 8 mg/5 ml, 4 mg/5 ml, tablets and soluble tablets (both with 8 mg Bromohexine) and solution for oral use 10 mg/5 ml, adapted to the need of the patients. The posology varies with the age and weight, but there are products for all age groups from infant on.

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Bromhexine is well absorbed and tolerated. Sometimes it is replaced by its metabolite Ambroxol, as in Mucosolvan or Mucoangin.

Sulbactam

Sulbactam is a β-lactamase inhibitor. This drug is given in combination with β-lactam antibiotics to inhibit β-lactamase, an enzyme produced by bacteria that destroys the antibiotics. A combination product of the above two drugs is being marketed under the brand name of Astarest in India.Since there were no methods available for the simultaneous estimation of the above two drugs in the combination product when we started our work. We attempted the same and successfully developed and validated a RP-HPLC method for this purpose.The work done on this method is incorporated in this chapter. The analytical methods reported so far are either in single or in combination with other drugs are reviewed in the following literature survey.

Literature Survey

Susmitha *et al*. (2013) reported five spectrophotometric methods for the determination of Bromhexine hydrochloride, validated and applied for the assay of the drug in

pharmaceuticals. Amit kumar *et al*. (2011) reported the estimation of Bromhexine (BH) in combination with Terbutaline. Sonawane *et al*. Reported a reverse phase high pressure liquid chromatographic (RP-HPLC) method for the simultaneous estimation of Amoxicillin trihydrate and Bromhexine hydrochloride from oily suspension. Madhura dhoka *et al*. (2010), reported a high performance liquid chromatographic method for the simultaneous determination of Amoxicillin trihydrate and Bromhexine hydrochloride in oral dosage forms. Rajan *et al*. (2013) reported a high performance liquid chromatography method for the simultaneous determination of Amoxicillin trihydrate and Bromhexine hydrochloride from the combine formulation i.e. capsules.

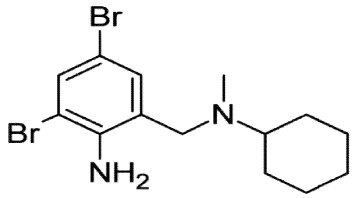

Fig. 1. Chemical Structure of Bromhexine

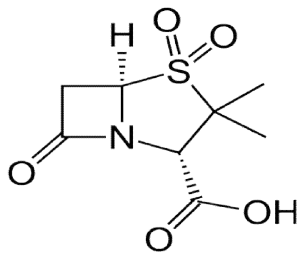

Fig. 2. Chemical Structure of Sulbactam

Satyanarayana *et al.* (2012) reported a reverse phase high-performance liquid chromatographic method for the simultaneous estimation of Terbutaline and Bromhexine in bulk and pharmaceutical dosage forms. Senthilraja *et al.* (2011) reported a reversed phase HPLC method for the simultaneous quantification of Terbutaline sulphate, Bromhexine hydrochloride and Guaifenesin in a cough syrup formulation. Madhukar *et al.* (2011), reported a reverse phase high performance liquid chromatographic method for the simultaneous estimation of Ampicillin sodium and Sulbactam sodium in injectable formulation. Dhandapani *et al*. (2010), reported a reverse phase liquid chromatographic method for simultaneous determination of Cefoperazone and Sulbactam in parenteral preparation. Palanikumar *et al*. (2010) reported an isocratic liquid chromatographic method with UV detection at 230 nm for the simultaneous determination of Ceftriaxone sodium and Sulbactam sodium in Cetriax□s 1.5gm injection. Anusha *et al*. (2012), reported a method for determining the concentration of Amoxicillin and Sulbactam simultaneously in Amoxirum forte injection vial, based on high-performance liquid chromatography. Sanjay Mohan *et al*. (2004), reported an isocratic liquid chromatographic method with UV detection at 220 nm is for the simultaneous determination of Ceftriaxone sodium and Sulbactam sodium in Sulbactomax. Masoom Raza *et al*. (2009), reported a method to determine Ceftriaxone and Sulbactam simultaneously in spiked plasma and combined formulations. Patel *et al*. (2012), Developed two UV spectrophotometric methods which are validated for the simultaneous determination of Cefuroxime sodium (CEF) and Sulbactam sodium (SUL) in their combined dosage forms. Pai *et al*. (2009), reported a reverse phase liquid chromatographic method for the simultaneous determination of Salbutamol sulphate and Bromhexine hydrochloride.

Aim and Objective

Various UV and HPLC methods were reported in the literature for the estimation of Bromhexine and Sulbactam individually and in-combination with other drug. According to literature survey there is no method reported for the simultaneous estimation of Bromhexine and Sulbactam by RP-HPLC in combined pharmaceutical dosage forms. So we planned to develop and validate a RP-HPLC method for simultaneous estimation of Bromhexine and Sulbactam in pharmaceutical dosage forms in accordance with the ICH guidelines.

The main aim and objective of the present study is

- To develop a new reverse phase high performance liquid chromatographic method for the simultaneous determination of Bromhexine and Sulbactam in pharmaceutical dosage form.
- To validate the developed method for the following parameters
  - System suitability
  - Specificity
  - Linearity
  - Accuracy
  - Precision
  - Limit of Detection
  - Limit of Quantification
  - Robustness
  - Solution stability.

EXPERIMENTAL PROCEDURE

Instrumentation: Chromatography was performed with Alliance Waters 2695 HPLC provided with high speed auto sampler, column oven, degasser and & 2996 PDA detector to provide a compact and with class Empower-2 software.

Reagents and chemicals: The reference samples of Bromhexine and Sulbactam were provided as gift samples from Spectrum pharma research solutions, Hyderabad. HPLC grade acetonitrile, HPLC grade methanol and all other chemicals were obtained from Merck chemical division, Mumbai. HPLC grade water obtained from Milli-Q water purification system was used throughout the study. Commercial formulations (Brand Name: Astarest; Lable Claim: Bromhexine and Sulbactam - 4 mg &2 mg /5ml) were purchased from the local pharmacy.

Preparation of buffer Solution: Accurately weighed 1.41gm of sodium dihyrogen ortho phosphate in a 1000ml of volumetric flask, about 900ml of HPLC grade water was added, sonicated and degassed and finally made up the volume to 1000ml with water, and then pH was adjusted to 4 with dilute orthophosphoric acid solution.

Preparation of diluent solution: Milli-Q water was used as diluent which is filtered through 0.22 micron filter.

Preparation of Standard Stock Solution: Standard stock solutions were prepared by dissolving 10mg of

Bromhexine and 5mg of Sulbactam into a clean and dry 25ml volumetric flask, to that 15 ml of diluent was added, sonicated for 5 minutes and volume was made up to 25 ml with the diluent to get stock solution with a concentration of 0.4mg/ml for Bromhexine and 0.2mg/ml for Sulbactum respectively.

Preparation of Working Standard Solutions: Aliquots of 0.25, 0.5, 0.75, 1, 1.25 & 1.5 ml were pipetted out from stock solution and transferred into 10 ml volumetric flask and the volume was made up to 10 ml with diluent. This gives the solutions of 10, 20, 30, 40, 50 and 60µg/ml for Bromhexine and 5, 10, 15, 20, 25 and 30 µg/ml for Sulbactam respectively.

Sample preparation: 5 bottles of Astarest syrup was transferred into a clean & dried beaker and mixed well. From the above syrup, 5 ml was transferred into a 100ml volumetric flask, 70ml of diluent was added and sonicated for 30 min, further the volume was made up with diluent and filtered. This solution gave 40µg/ml of Bromhexine and 20µg/ml of Sulbactum respectively.

Chromatographic conditions: The chromatographic separation was carried out under the isocratic conditions. Chromatographic separation was achieved by injecting a volume of 10µl of standard into Altima (150x 4.6mm, 5µm) column. The mobile phase of composition 400 ml of buffer (pH 4) and 600ml of acetonitrile were allowed to flow through the column at a flow rate of 1.0 ml/min for a period of 6min at 30^0C column temperature. Detection of the component was carried out at a wavelength of 260 nm. The retention time of the components were found to be 2.1 and 3.2 min for Bromhexine and Sulbactam respectively.

Method Validation

System Suitability Tests: Data from six injections of 10 µl of the working standard solutions of Bromhexine (40µg) and Sulbactam (20µg) were used for the evaluation of the system suitability parameters like tailing factor, the number of theoretical plates, retention time and resolution factor.

Specificity

The specificity of the method was performed by injecting blank solution, placebo solution and standard solutions of Bromohexine and Sulbactam separately.

Linearity: By taking appropriate aliquots of the standard Bromhexine and Sulbactam solutions with the mobile phase, six working solutions ranging between 10-60 µg/ml and 5-30 µg/ml were prepared. Each experiment linearity point was performed in triplicate according to optimized chromatographic conditions. The peak areas of the chromatograms were plotted against the concentration of Bromhexine and Sulbactam to obtain the calibration curve.

Accuracy: Previously analyzed samples of Bromhexine (40µg) and Sulbactam (20µg) to which known amounts of standard Bromhexine and Sulbactam corresponding to 50%, 100% and 150% of target concentration were added and analyzed in triplicate. The accuracy was expressed as the percentage of analyte recovered by the proposed method.

Precision: The repeatability and intermediate precision were determined by analyzing the six samples of Bromhexine (40µg) and Sulbactam (20µg).

Limit of detection and the Limit of quantification: Limit of detection (LOD) and limit of quantification (LOQ) of Bromohexine and Sulbactam were determined by calibration curve method. Solutions of both Bromohexine and Sulbactam were prepared in linearity range and injected in triplicate. Average peak area of three analyses was plotted against concentration. LOD and LOQ were calculated by using following equations.

LOD = (3.3 ×Syx)/b, LOQ= (10.0×Syx)/b.

Where Syx is residual variance due to regression; b is slope.

Robustness: The robustness of the method was performed by deliberately changing the chromatographic conditions. The parameters included slight variation in organic phase percentage in the mobile phase (54, 66%), flow rate (0.9, 1.1 ml/min) and column temperature (25, 35°C).

Stability: The sample solutions of Bromhexine (40µg) and Sulbactam (20µg) were injected at 0 hrs (comparison sample) and after 24 hrs (stability sample) by keeping at ambient room temperature. Stability was determined by determining %RSD for sample and standard solutions.

RESULTS AND DISCUSSION

Method Development

Initially reverse phase liquid chromatography separation was attempted using various ratios of methanol and water, acetonitrile and water as mobile phases, in which both the drugs did not responded properly, and the resolution was also poor. Further several systematic trials were performed to optimize the mobile phase and the organic content of mobile phase was also investigated to optimize the separation of both drugs. To improve the tailing factor, the pH of mobile phase becomes important factor. The organic content of mobile phase was investigated further to optimize the separation of both drugs. To improve the tailing factor, the pH of mobile phase becomes important factor. Thereafter, buffer: acetonitrile were taken in ratio of 40:60%v/v and with a flow rate of 1.0 ml/min was employed. Preliminary development trials were performed with octdecyl columns of different types, configurations and from different manufacturers.

Finally Altima column (4.6x150mm, 5µm) was selected as the stationary phase to improve resolution and the tailing of both peaks were reduced considerably and brought close to 1. To analyze both drugs detection were tried at various wavelengths from 205nm to 280nm. Both Bromhexine and Sulbactam showed maximum absorption at 260nm of wavelength and the same was selected as the detection wavelength for PDA detector. The retention times were found to about 2.1 min and 3.2 min for Bromhexine and Sulbactam respectively. The chromatograms obtained for blank injection, placebo injection and optimized method were shown in the Fig.3, 4 and 5 respectively and optimized chromatographic conditions were shown in Table 1.

Method Validation

System Suitability: System suitability parameters such as number of theoretical plates, peak tailing, retention time and resolution factor were determined.

Table 1. Optimized chromatographic conditions

S. No.	Parameter	Condition
1	Mobile phase	Buffer: Acetonitrile 40:60%v/v
2	pH	4
3	Diluent	Water
4	Column, make	Altima C18, 150 x 4.6 mm, 5µm
5	Column temperature	30^0C
6	Wave length	260nm
7	Injection volume	10µl
8	Flow rate	1.0ml/min
9	Run time	6 min
10	Retentiontime (Bromhexine)	2.1 min
11	Retentiontime (Sulbactam)	3.2 min

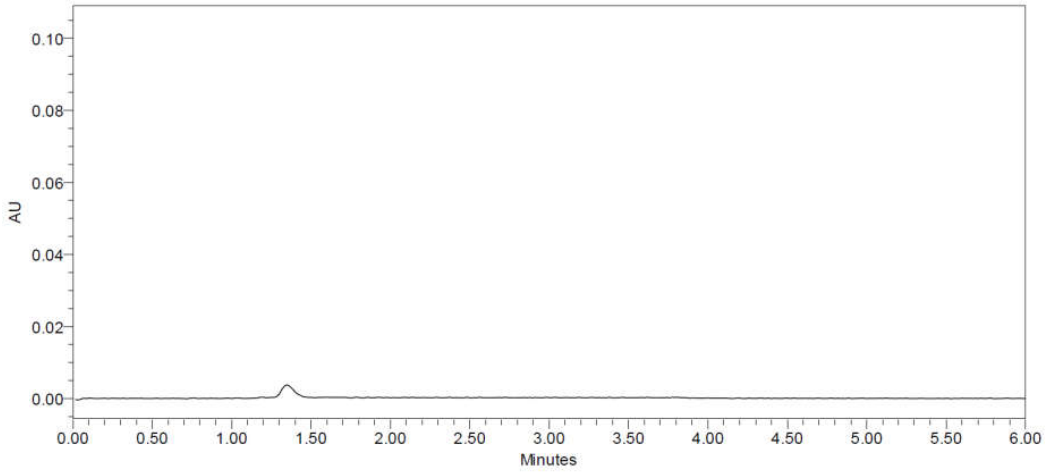

Fig. 3. Chromatogram of Blank

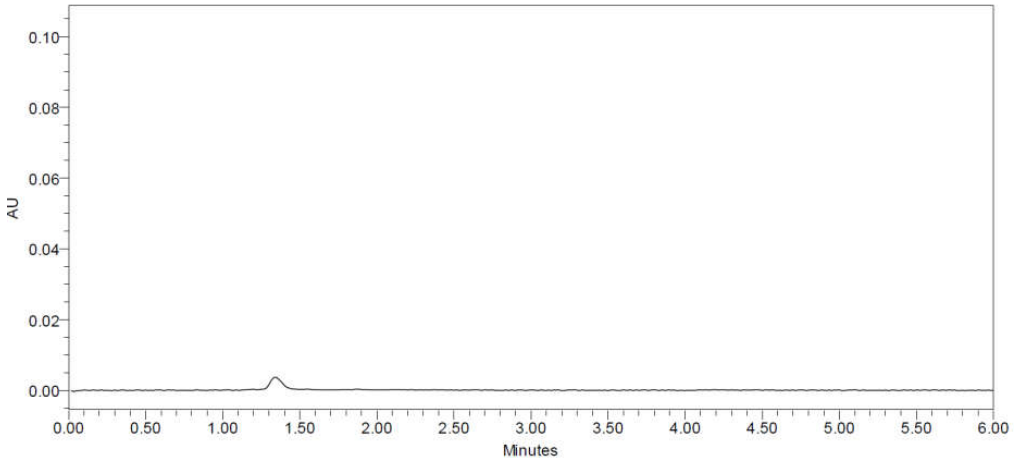

Fig. 4. Chromatogram of Placebo

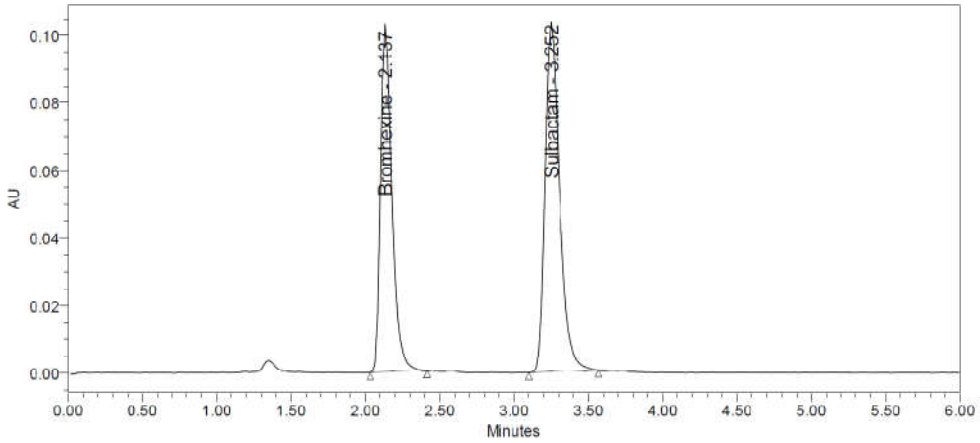

Fig. 5. Chromatogram of Bromhexine and Sulbactam standards

Table 2. System Suitability of Bromhexine and Sulbactam

	Bromhexine			Sulbactam		
S.No	Area	USP Plate Count	USP Tailing	Area	USP Plate Count	USP Tailing
1	540304	3958	1.35	700744	5478	1.31
2	534168	3711	1.37	707053	5420	1.32
3	538333	3938	1.39	697362	5457	1.33
4	537979	3657	1.37	704753	5445	1.34
5	541389	3696	1.38	700069	5455	1.32
6	540712	3774	1.37	694282	5438	1.32
Mean	538814			700711		
Std. Dev.	2644.50			4680.17		
% RSD	0.5			0.7		

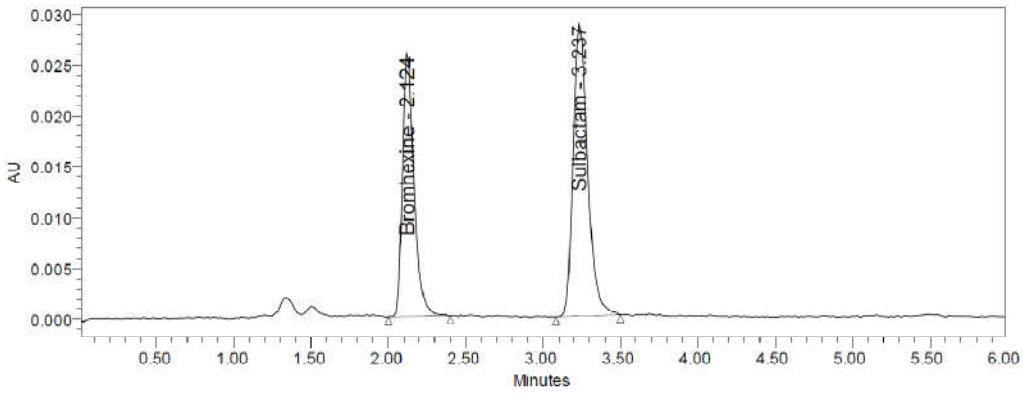

Fig. 6. Linearity 25% chromatogram of Bromhexine and Sulbactam

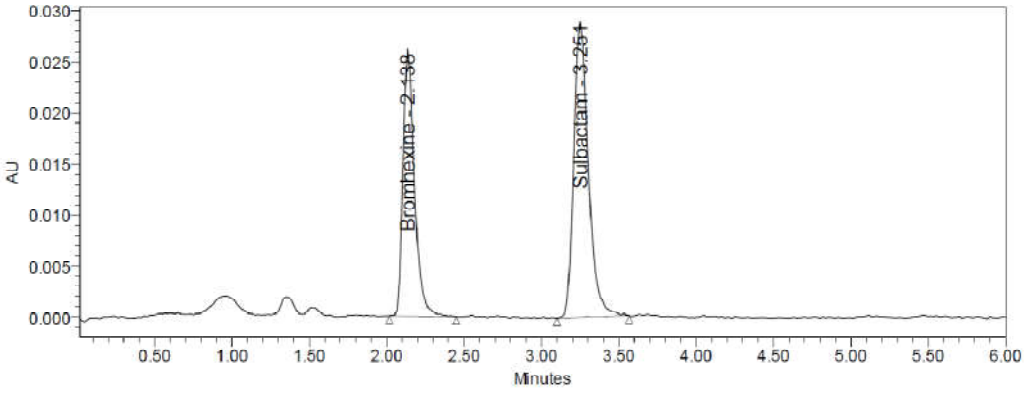

Fig. 7. Linearity 50% chromatogram of Bromhexine and Sulbactam

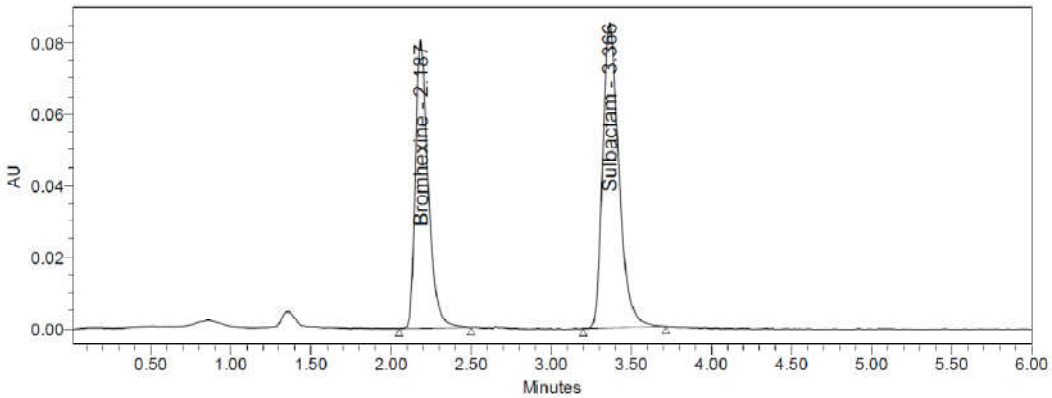

Fig. 8. Linearity 75% chromatogram of Bromhexine and Sulbactam

The total run time required for the method is only 6 minutes for eluting both Bromhexine and Sulbactam. The results obtained were shown in Table No.2. The number of theoretical plates was found to be > 2000, USP tailing was < 2 and USP resolution is above 2. The % RSD of areas for Bromhexine and Sulbactam were 0.5 and 0.7 respectively.

Specificity

The specificity of the method was performed by injecting blank solution, placebo solution and standard solutions separately. The chromatogram of the drug was compared with blank and placebo chromatogram to verify the interference.

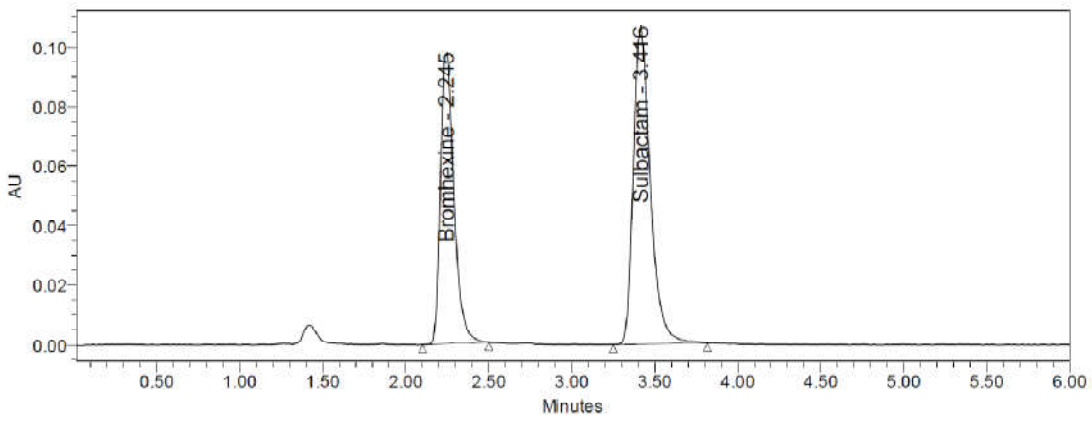

Fig. 9. Linearity 100% chromatogram of Bromhexine and Sulbactam

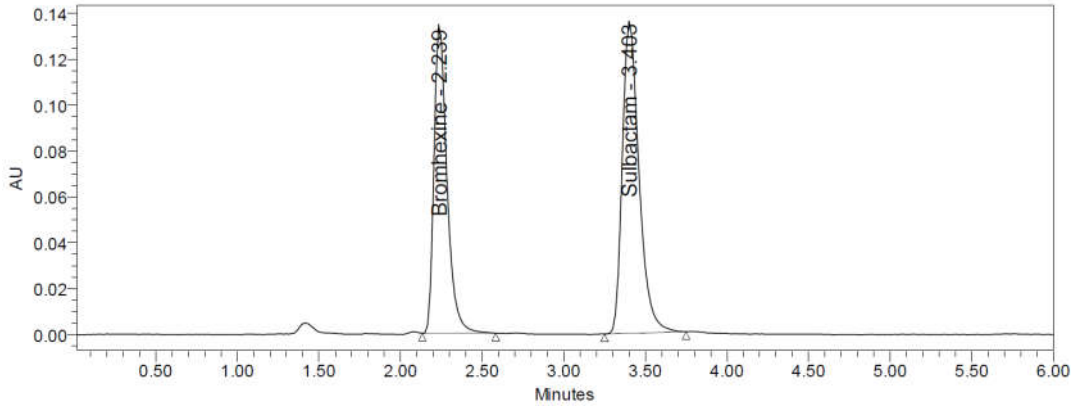

Fig.10. Linearity 125% chromatogram of Bromhexine and Sulbactam

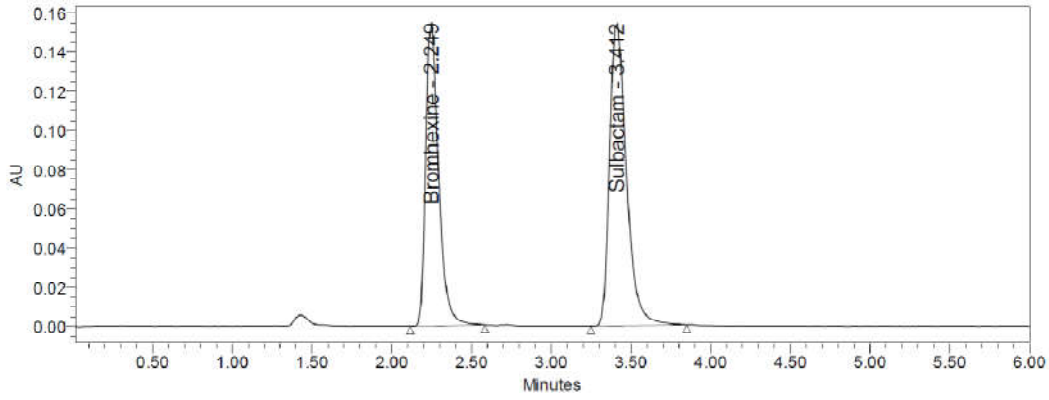

Fig.11. Linearity 150% chromatogram of Bromhexine and Sulbactam

No interfering peak was observed at the retention time of Bromhexine and Sulbactam. Hence, the method is specific for the determination of Bromhexine and Sulbactam.

Linearity

Bromhexine showed a linearity of response between 10-60 µg/ml and Sulbactam showed a linearity of response between 5-30 µg/ml. These were represented by a linear regression equation as follows: y (Bromhexine)= 13746x + 619.65 (r^2=0.9999), y(Sulbactam)= 38096x + 2441 (r^2=0.9997) and regression line was established by least squares method and correlation coefficient (r^2) for Bromhexine and Sulbactam is found to be greater than 0.98. Hence, the curves established were linear. The results were shown in the Table 3 and Fig. 6-13.

Accuracy

To the pre analyzed sample solution, a definite concentration of standard drug (50%, 100% & 150 % level) was added and recovery was studied. The % mean recovery for Bromhexine and Sulbactam are 99.97% and 100.06%, respectively and these results are within acceptable limit of 98-102. The % RSD for Bromhexine and Sulbactam are 1.1 and 0.9 respectively and %RSD for Bromhexine and Sulbactam is within limit of ≤2. Hence, the proposed method is accurate and the results are summarized in Table-4 and Figure 14-16.

Precision: The repeatability and Intermediate precision data were summarized in Table 5 and 6, respectively and were assessed by the use of standard solutions of Bromohexine and Sulbactam.

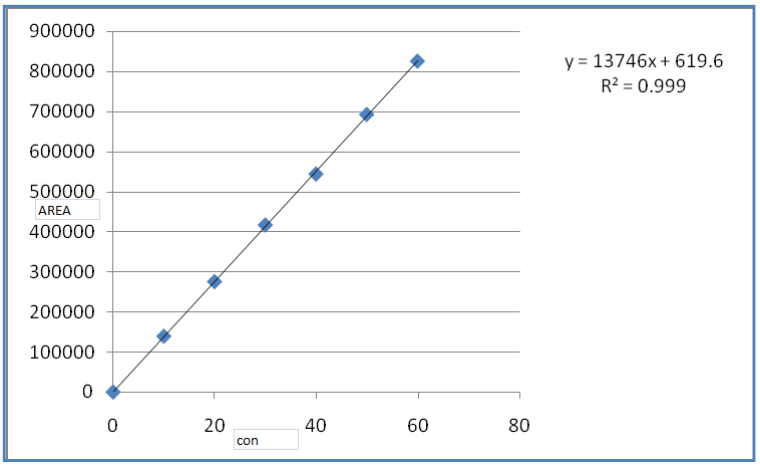

Fig.12. Calibration Curve for Bromhexine

Table 3. Linearity data of Bromhexine and Sulbactam

Bromhexine		Sulbactam	
Conc. (µg/ml)	Peak area Average(n=3)	Conc. (µg/ml)	Peak area Average(n=3)
10	139020	5	188298
20	275225	10	393333
30	416368	15	573983
40	543543	20	758611
50	691987	25	964101
60	824893	30	1138861

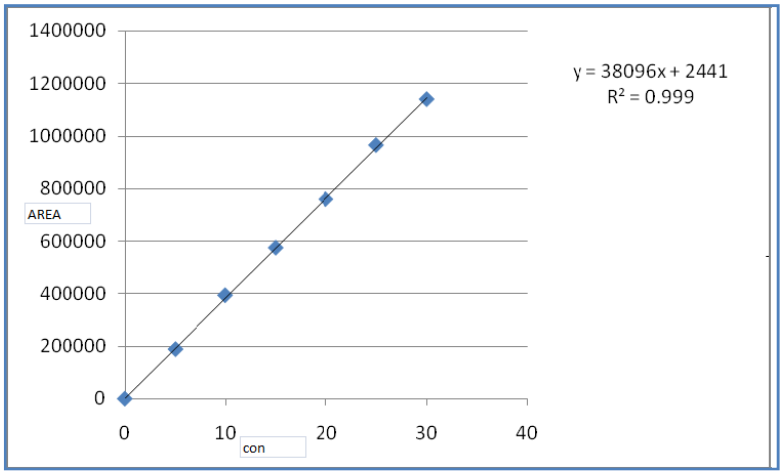

Fig.13. Calibration Curve for Sulbactam

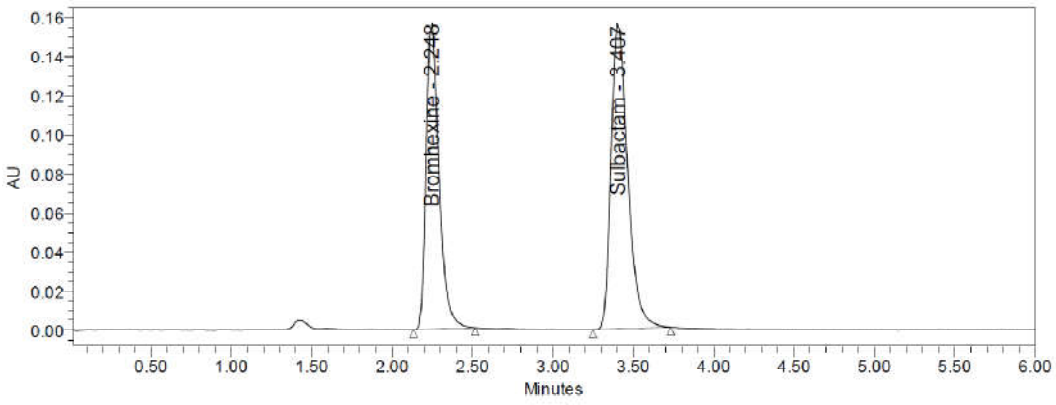

Fig. 14. Accuracy 50% chromatogram of Bromhexine and Sulbactam

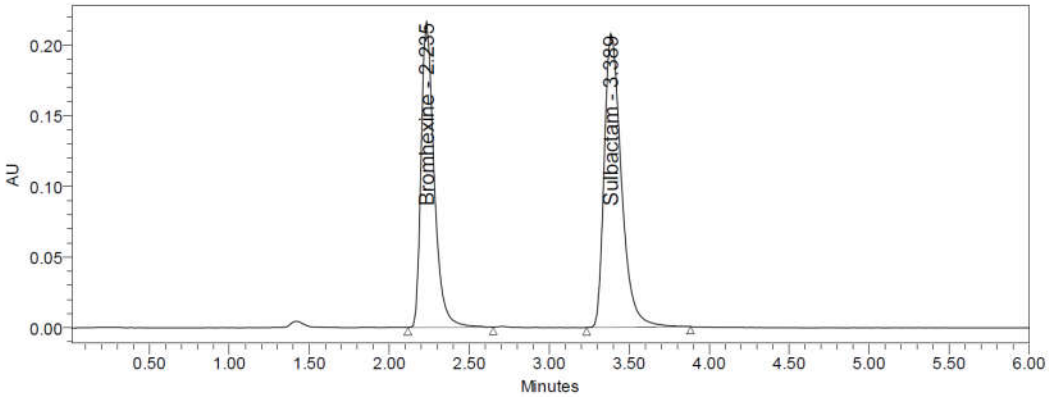

Fig. 15. Accuracy 100% chromatogram of Bromhexine and Sulbactam

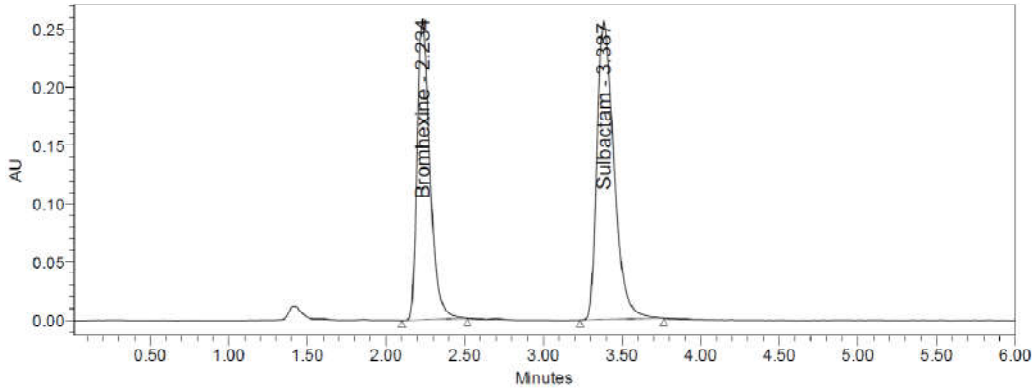

Fig.16. Accuracy 150% chromatogram of Bromhexine and Sulbactam

Table 4. Results of Recovery Experiments of Bromhexine and Sulbactam

Preanalysed amount (µg/ml)		Spiked Amount (µg/ml)		% Recovered	
Bromhexine	Sulbactam	Bromhexine	Sulbactam	Bromhexine	Sulbactam
40	20	20	10	100.93	100.31
40	20	20	10	100.78	99.06
40	20	20	10	99.33	99.56
40	20	40	20	99.48	98.66
40	20	40	20	100.92	100.62
40	20	40	20	98.92	100.9
40	20	60	30	98.19	101.05
40	20	60	30	99.69	100.71
40	20	60	30	101.46	99.63
			MEAN	99.97	100.06
			SD	1.1	0.86
			%RSD	1.1	0.9

Table 5. Results of Repeatability of Bromhexine and Sulbactam

	Bromhexine			Sulbactam		
S.No	Area	USP Plate Count	USP Tailing	Area	USP Plate Count	USP Tailing
1	542957	4023	1.35	706362	5418	1.34
2	536424	3780	1.37	703018	5265	1.34
3	539519	3707	1.39	706813	5520	1.31
4	541519	3739	1.37	706899	5597	1.33
5	544129	3678	1.36	693735	5631	1.32
6	534132	4056	1.39	696628	5616	1.32
Mean	539780			702243		
Std. Dev.	3879.74			5727.90		
% RSD	0.7			0.8		

Repeatability: Six replicates injections in same concentration of Bromhexine and Sulbactam were analyzed in the same day for repeatability and the % RSD for Bromhexine and Sulbactam found to be 0.7 and 0.8 respectively and % RSD for Bromhexine and Sulbactam found to be within acceptable limit of ≤2.

Intermediate Precision: Six replicates injections in same concentration were analyzed on two different days with different analyst and column for verifying the variation in the precision and the % RSD for Bromhexine and Sulbactam is found to be 0.3 and 0.7 respectively and it is within acceptable limit of ≤2.

Table 6. Results of Intermediate precision of Bromhexine and Sulbactam

	Bromhexine			Sulbactam		
S.No	Area	USP Plate Count	USP Tailing	Area	USP Plate Count	USP Tailing
1	542250	3954	1.36	710189	5458	1.33
2	539210	3698	1.38	714565	5405	1.34
3	542891	3930	1.40	704596	5439	1.35
4	542022	3649	1.39	708787	5436	1.35
5	540281	3698	1.38	705537	5439	1.33
6	544444	3768	1.39	699836	5421	1.33
Mean	541850			707252		
Std. Dev.	1867.27			5088.32		
% RSD	0.3			0.7		

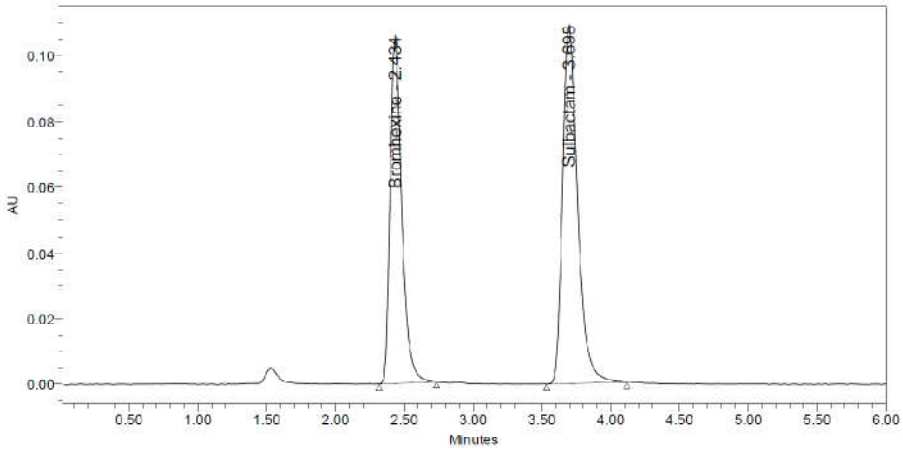

Fig.17. Robustness (Flow minus: 0.9ml/min) chromatogram of Bromhexine and Sulbactam

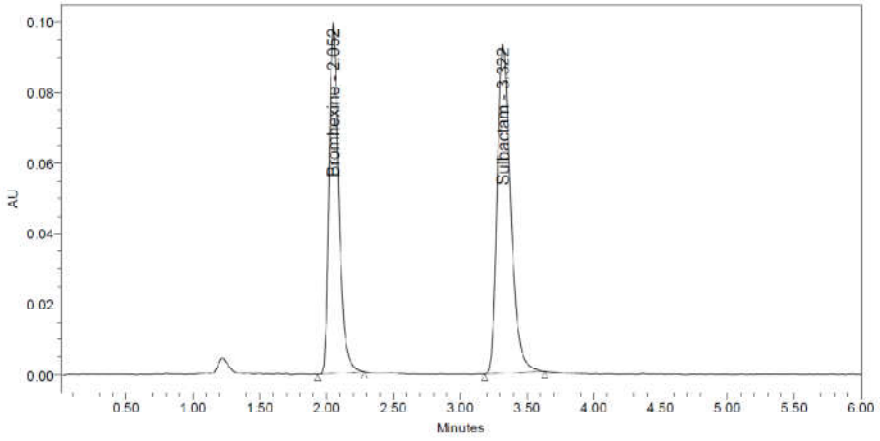

Fig.18. Robustness (Flow Plus: 1.1ml/min) chromatogram of Bromhexine and Sulbactam

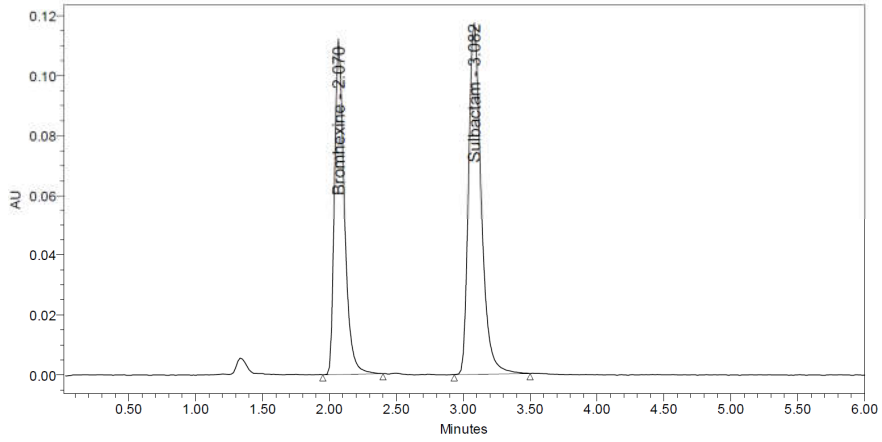

Fig. 19. Robustness (Mobile Phase Minus: 54%) chromatogram of Bromhexine and Sulbactam

Hence, the method is reproducible on different days with different analyst and column. This indicates that the method is precise.

Robustness: To evaluate the robustness of the developed HPLC method, few chromatographic conditions were deliberately altered.

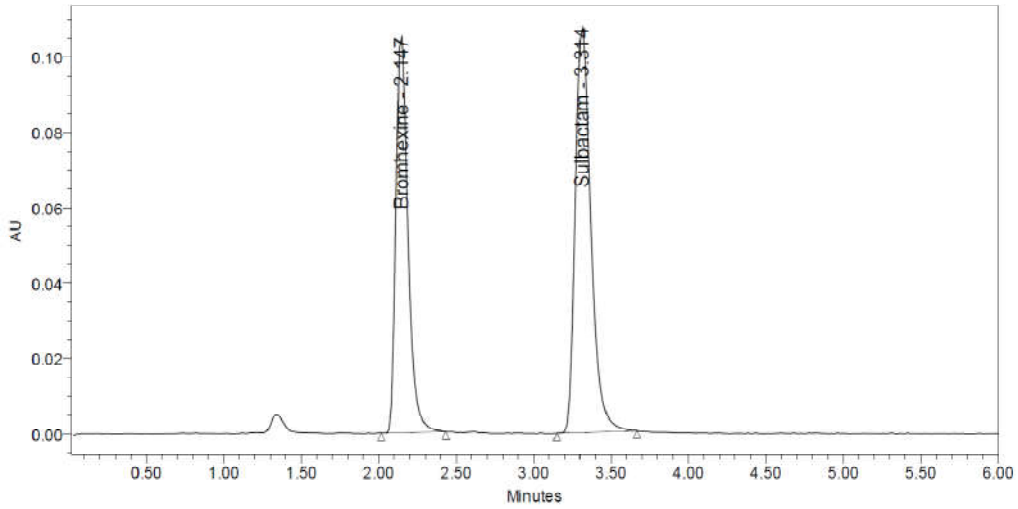

Fig. 20. Robustness (Mobile Phase Plus: 66%) chromatogram of Bromhexine and Sulbactam

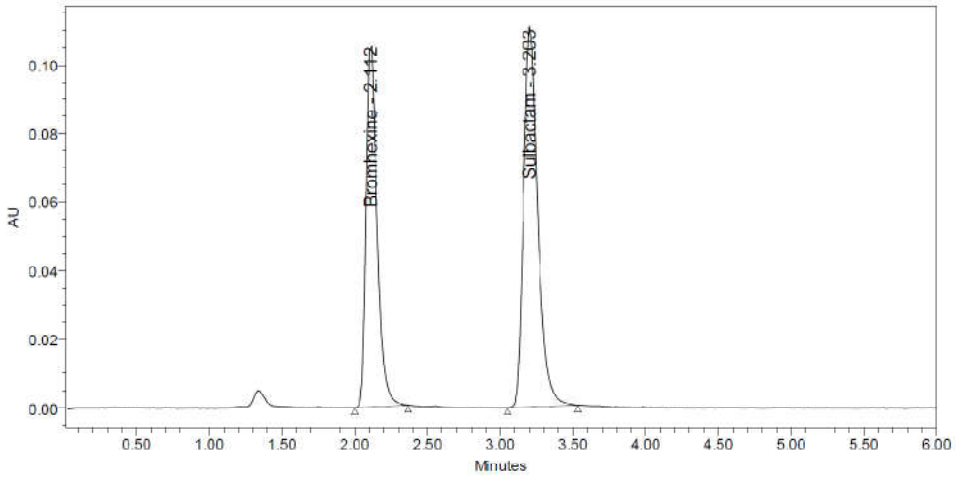

Fig.21. Robustness (Temperature Minus: 25°C) chromatogram of Bromhexine and Sulbactam

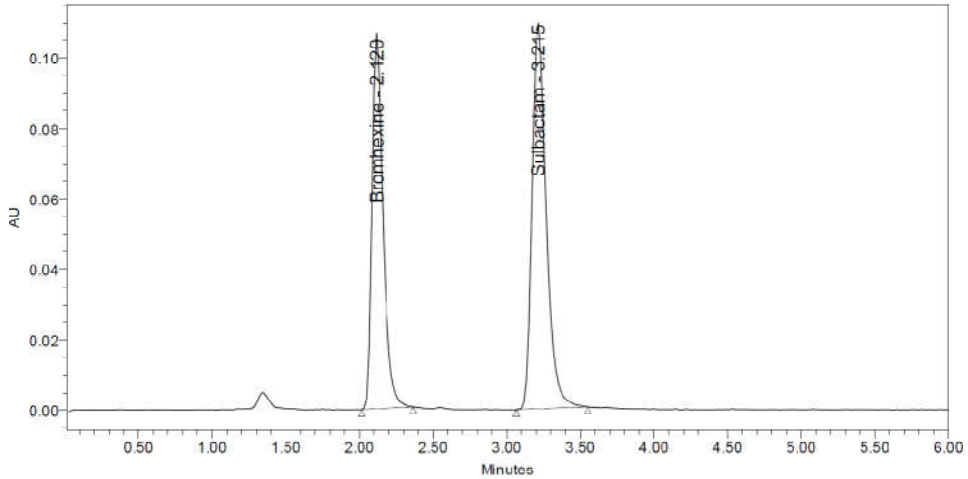

Fig. 22. Robustness (Temperature Plus: 35°C) chromatogram of Bromhexine and Sulbactam

The robustness was established by changing the flow rate, column temperature and composition of the mobile phase within allowable limits from actual chromatographic conditions. It was observed that there were no marked change in mean R_t and RSD is within limit of ≤2. The tailing factor, resolution factor and no. of theoretical plates were found to be acceptable limits for both Bromhexine and Sulbactam. Hence, the method is reliable with variations in the analytical conditions and the results are shown in Table No.8.7 and Figure No. 17-22.

Stability of sample solution: The sample solution injected after 24 hrs by keeping at ambient room temperature 30^0C did not show any appreciable change. The deviation in the assay is not more than 2 and the results are shown in Table-8.

LOD and LOQ: LOD and LOQ for Bromhexine were 0.04 and 0.11 µg/ml, respectively and for Sulbactum were 0.09 and 0.27 µg/ml respectively.

Table 7(a). Robustness – Flow Minus (n=6)

S.No.	Parameter	Bromhexine	Sulbactam
1.	% RSD of area	0.5	0.3
2.	Tailing Factor	1.36	1.34
3.	Plate count	3987	5772

Table 7(b). Robustness- Flow Plus (n=6)

S.No.	Parameter	Bromhexine	Sulbactam
1.	% RSD of area	0.4	0.5
2.	Tailing Factor	1.32	1.31
3.	Plate count	3969	5398

Table-7(c). Robustness - Mobile Phase Minus (n=6)

S.No.	Parameter	Bromhexine	Sulbactam
1.	% RSD of area	0.5	1.0
2.	Tailing Factor	1.36	1.34
3.	Plate count	3972	5464

Robustness – Mobile Phase Plus (n=6)

S.No.	Parameter	Bromhexine	Sulbactam
1.	% RSD of area	0.7	0.6
2.	Tailing Factor	1.38	1.32
3.	Plate count	4058	5571

Table- 7(e). Robustness- Temperature Minus (n=6)

S.No.	Parameter	Bromhexine	Sulbactam
1.	% RSD of area	0.8	0.6
2.	Tailing Factor	1.36	1.32
3.	Plate count	4056	5597

Table-7(f). Robustness – Temperature Plus (n=6)

S.No.	Parameter	Bromhexine	Sulbactam
1.	% RSD of area	0.9	0.4
2.	Tailing Factor	1.35	1.33
3.	Plate count	3961	5501

Table 8. Stability data of Bromhexine and Sulbactam

Drug	%Assay at 0 hr*	%Assay at 24hr*	Deviation
Bromhexine	99.78	98.31	1.04
Sulbactam	99.82	99.11	0.50

* n=6 for each parameter

The lowest values of LOD and LOQ as obtained by the proposed method indicate that the method is sensitive and the results are shown in Table-9.

Table 9. LOD and LOQ data of Bromhexine and Sulbactam

	Bromhexine			Sulbactam	
S.no	Slope	Y-intercept	S.no	Slope	Y-intercept
1	13850	504.6	1	38383	1264
2	13715	564.4	2	38224	3135
3	13673	789.9	3	37681	2922
AVG	13746	619.6	AVG	38096	2440
SD		150.46	SD		1024.29
LOD		0.04	LOD		0.09
LOQ		0.11	LOQ		0.27

Assay: The percentage assay of labeled claim of Bromhexine and Sulbactam present in the astarest syrup were 99.78±0.72 % and 99.80±0.82% respectively. RSD values for both Bromhexine and Sulbactam are within limit of ≤2 and the results were shown in Figure No. 23 and Table 10.

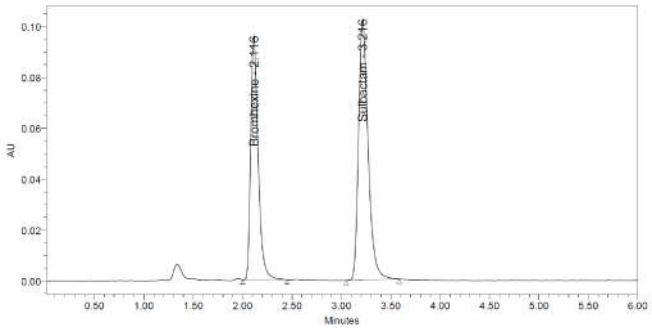

Fig.23. Assay chromatogram of Bromhexine and Sulbactam

Table 10. Assay of pharmaceutical dosage form

S. No.	Drug Name	Amount injected (µg/ml)	Amount found (µg/ml)	% Assay ± SD*
1	Bromhexine	40	39.91	99.78±0.72
2	Sulbactam	20	19.96	99.80±0.82

* n=6 for each parameter; Label Claim: 4mg Bromhexine+2mg of Salbactum/5 ml

Conclusion

A new simple, precise and accurate HPLC method was developed and validated for the simultaneous estimation of Bromhexine and Sulbactam in pharmaceutical dosage form. In this method, Altima C18 (150 x 4.6 mm, 5µm) column was selected as the stationary phase. Phosphate buffer (pH 4), acetonitrile were taken in the ratio 40:60%v/v and used as mobile phase at a flow rate of 1.0 ml/min. The retention times of Bromhexine and Sulbactam were found to be 2.1 min & 3.2 min, respectively. This HPLC method for the determination of Bromhexine and Sulbactam was validated as per the ICH guidelines. In this method, the numbers of theoretical plates were above 2000, tailing factor is less than 2 and RSD of peak area is less than 2, this indicates that the optimized method met the system suitability parameters. The regression coefficient value (r^2) was 0.999 for Bromhexine and Sulbactam and the response was linear.

The percentage mean recovery of Bromhexine and Sulbactam were found to be 99.97% and 100.06%, respectively and it showed that the proposed method is accurate. RSD values of repeatability and intermediate precision were ≤2 and the method is precise. The lowest values of LOD and LOQ as obtained by the proposed HPLC method indicate that the method is sensitive. The solution stability studies of method indicate that the Bromhexine and Sulbactam drugs were stable up to 24 hours. In robustness chromatographic conditions were changed as flow minus: 0.9 ml/min; flow plus: 1.1ml/min; temperature minus: 25^0C; temperature plus: 35^0C; mobile phase minus: organic phase 54%v/v; mobile phase plus: organic phase 66%v/v. These changes didn't show any variation in results and it showed the reliability of the method. Hence, the developed method can be successfully employed for routine quality control of Bromhexine and Sulbactam in drug testing laboratories and pharmaceutical industries.

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