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Research Article

DEVELOPMENT OF A VALIDATED METHOD FOR THE SIULTANEOUS ESTIATION OF AMPICILLIN AND SULBACTAM IN BULK AND TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT

Objective: *A simple, economic, selective and precise RP-HPLC method has been developed and validated for Ampicillin and Sulbactam in bulk and tablet dosage form.*

Methods: *The isocratic LC analysis was performed on inertsil ODS C18 column (250 mm x 4.6 mm, 5 µ) using mobile phase composed of Methanol and 0.01M Tetrabutyl ammonium hydroxide (40:60 v/v) at a flow rate of 1.2 ml/min. Quantitation was performed using UV detector at 240 nm.*

Results: *The retention time was found to be 3.541min for Ampicillin and 4.625min for Sulbactum. The analytical method was validated according to ICH guidelines. The linearity was observed in the range of 20-80 µg/ml for Ampicillin and 20-80 µg/ml for Sulbactum with correlation coefficient r2=0.999 for both Ampicillin and Sulbactam. The % recovery was found to be 99.83 - 100.16 % for both ampicillin and sulbactam. The relative standard deviation values for repeatability and intermediate precision studies were less than 2%.*

Conclusion: *The proposed method was precise, rapid, accurate, and cost-effective and can be used for the routine estimation for ampicillin and sulbactam in tablet dosage form.*

KEYWORDS: *Ampicillin, RP-HPLC, Sulbactam, Tablet dosage.*

INTRDUCTION

Ampicillin is chemically (2S,5R,6R)-6-([(2R)-2-amino-2-phenylacetyl]amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]heptane-2- carboxylic acid. It is an Anti-Bacterial agent and it binds to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall, Ampicillin inhibits the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that Ampicillin interferes with an autolysin inhibitor.

Sulbactum is chemically (2S,5R)-3,3-Dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide. It is a β-lactamase inhibitor. Sulbactam is an irreversible inhibitor of β-lactamase. it binds to the enzyme and does not allow it to degrade the antibiotic.

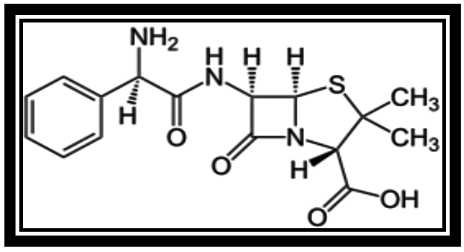

Fig.1: Chemical Structure of Ampicillin

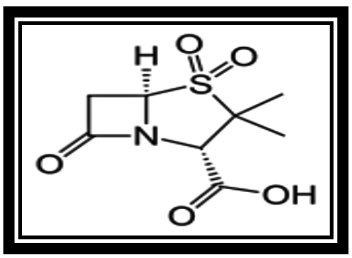

Fig.2: Chemical Structure of Sulbactum

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MATERIALS AND METHODS

Instruments:

- HPLC –Waters Model NO.2690/5 series Compact System Consisting of Inertsil- C18 ODS column.
- Electronic balance (SARTORIOUS)
- Sonicator (FAST CLEAN)

Chemicals:

- Methanol- HPLC Grade.
- Tetrabutyl ammonium hydroxide- HPLC Grade.

Raw Material:

Ampicillin and Sulbactam Working Standards were obtained as a gift samples from CIPLA PHARMACEUTICALS, Mumbai, India.

Method development:

Optimized chromatographic conditions:

Stationary Phase : Inertsil ODS C18 (250 x 4.6mm, 5 µ)

Mobile Phase : Methanol : 0.01M Tetrabutyl ammonium hydroxide (40:60)

Flow rate (ml/min): 1.2 ml/min

Run time : 10min

Column Temperature : Ambient

Injection volume : 20 µl

Detection wavelength : 240nm

Preparation of Standard Solution:

Weigh accurately about 10mg of Ampicillin and Sulbactam working standards and dissolved in 10ml of Mobile phase and sonicated for 20 minutes (1000ppm) and 1 ml was taken from solution into a 10ml volumetric flask and diluted to 10 ml with mobile phase (100ppm) [1-3].

Method validation: [4-6]

System suitability:

A Standard solution was prepared by using Ampicillin and Sulbactam working standards as per test method and was injected Five times into the HPLC system.

The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Ampicillin and Sulbactam**,** retention times and peak areas.

Specificity:

Ampicillin and Sulbactam Solutions of standard and sample were prepared as per the test method are injected into chromatographic system.

Precision:

It is expected that an analytical method should generate outcomes that are reproducible. Precise analytical method leads to accurate results.

Repeatability:

a. System precision: Standard solution prepared as per test method and injected five times.

b. Method precision: Prepared six sample preparations individually using single as per test method and injected each solution.

Intermediate precision (analyst to analyst variability):

A study was conducted by two analysts as per test method

Accuracy:

Accuracy is a measure of the closeness of the experimental value to the actual amount of the substance in the matrix. Accuracy is to be established over the entire calibration range of the analytical method so that at any point of determination, results obtained would be reliable.

Accuracy was performed in triplicate concentrations as per test method with equivalent amount of Ampicillin and Sulbactam into each volumetric flask for each spike level to get the concentration of Ampicillin and Sulbactam equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of Ampicillin and Sulbactam were calculated.

Linearity:

Linearity is a key parameter of analytical method that demonstrates the limit within which the intended method is to be used for its optimum performance. A Series of solutions are prepared using Ampicillin and Sulbactam working standards at concentration levels from 20ppm to 80ppm of target concentration .Measure the peak area response of solution.

Ruggedness:

System to system variability:

System to system variability study was conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared and each was analyzed as per test method. Comparison of both the results obtained on two different HPLC systems, shows that the assay test method are rugged for System to system variability.

Robustness:

Robustness of analytical method is the ability of a method to resist the change in its performance in spite of small, deliberate change in method parameters. It is an important parameter of analytical method as a small, un-intentional change in method parameters like solvent composition, buffer strength, pH etc. may occur during routine use and may hamper the performance of said method. It is expected that such change should not alter the performance of the analytical method.

A study was conducted to determine the effect of variation in flow rate and mobile phase composition. Standard solutions was prepared as per the test method and was injected into the HPLC system using flow rates 1.0ml/min and 1.4ml/min and mobile phase compositions Methanol: 0.01M Tetra butyl ammonium hydroxide (45:55) and (35:65). The system suitability parameters were evaluated and found to be within the limits for both Ampicillin and Sulbactam.

LOD and LOQ

LOQ represents the lowermost concentration that can be analyzed with acceptable accuracy and precision. Generally, LOQ is the first calibration standard.

From the linearity data calculate the limit of detection and quantitation, using the following formula.

$$LOD = \frac{3.3\,\sigma}{S}$$

σ = standard deviation of the response
S = slope of the calibration curve of the analyte.

$$LOQ = \frac{10\,\sigma}{S}$$

σ = standard deviation of the response
S = slope of the calibration curve of the analyte.

RESULTS AND DISCUSSION

The simultaneous estimation of Ampicillin and Sulbactum was done by RP-HPLC and in the optimized method the mobile phase consists of Methanol and 0.01M Tetrabutyl ammonium hydroxide (40:60). Then finally filtered using 0.45µ membrane filter paper and degassed in sonicator for15 minutes. The detection is carried out using UV detector at 240 nm. The solutions are following at the constant flow rate of 1.2 ml/min. The retention time for Ampicillin and Sulbactum was 3.541 & 4.625 min respectively. Linearity ranges for Ampicillin and Sulbactum were 20 - 80µg/mL for both the drugs and the results were found in the acceptable as (R2) = 0.999 for both the drugs. LOD were 0.52µg/ml and0.83µg/ml and LOQ were 1.8µg/ml and 5.3µg/mL for Ampicillin and Sulbactum respectively. The all parameters value of RSD is less than 2.0% indicating the accuracy and precision of the method. The percentage recoveries were found 98.66 – 101.53% and 98.86 – 100.56% for Ampicillin and Sulbactum respectively.

Table No. 1: Results for System Suitability- Ampicillin

S No.	RT	Peak Area	USP Plate count	USP Tailing
1	3.225	2164732	6648	1.11
2	3.227	2161848	6673	1.14
3	3.220	2198427	6630	1.16
4	3.221	2231236	6778	1.14
5	3.222	2254490	6687	1.13
% RSD	0.02	1.84	0.91	0.52

Table No. 2: Results for System suitability- Sulbactum

S No.	RT	Peak Area	USP Plate count	USP Tailing
1	4.522	4037216	5368	1.49
2	4.522	4063397	5441	1.47
3	4.516	4115511	5413	1.48
4	4.519	4126557	5357	1.46
5	4.518	4195611	5398	1.50
% RSD	0.02	1.84	0.91	0.52

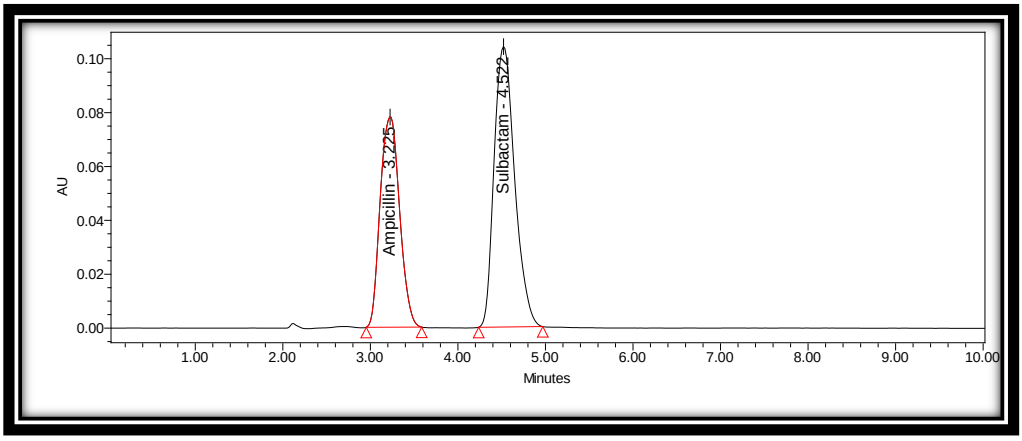

Fig. 3: Chromatograms for System suitability

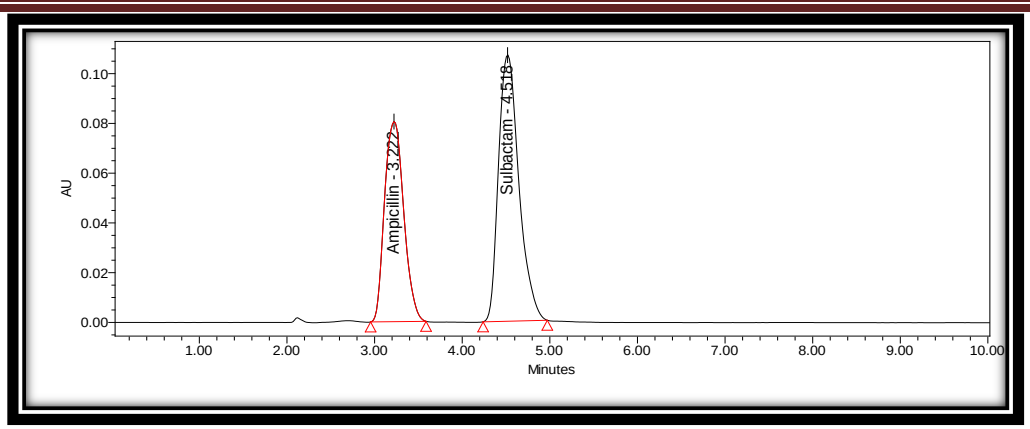

Fig. 4: Chromatogram for standard

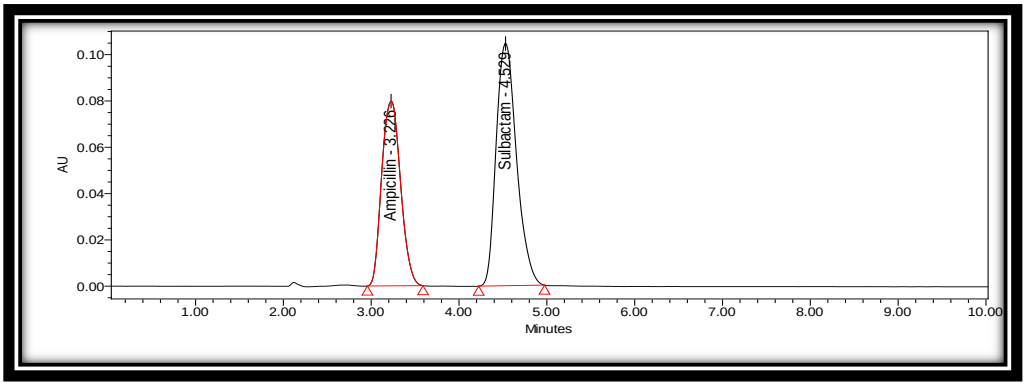

Fig. 5: Chromatogram for sample

Table No. 3: Results for System precision

S No.	Peak Area		% Assay	
	Ampicillin	Sulbactum	Ampicillin	Sulbactum
1	2164732	4037216	98.66	98.86
2	2161848	4063397	99.30	99.86
3	2198427	4115511	101.53	100.56
4	2231236	4126557	100.53	99.54
5	2254490	4195611	99.98	99.54
% RSD	1.84	1.50	1.10	0.63

Table No. 4: Results for Method precision

S No.	Peak Area		% Assay	
	Ampicillin	Sulbactum	Ampicillin	Sulbactum
1	2245703	4196762	99.55	98.54
2	2291408	4237539	99.88	99.58
3	2278639	4219201	99.40	98.86
4	2231236	4278401	100.30	99.56
5	2267407	4235847	100.53	99.86
6	2278639	4219201	99.28	99.06
% RSD	0.90	1.50	1.10	0.50

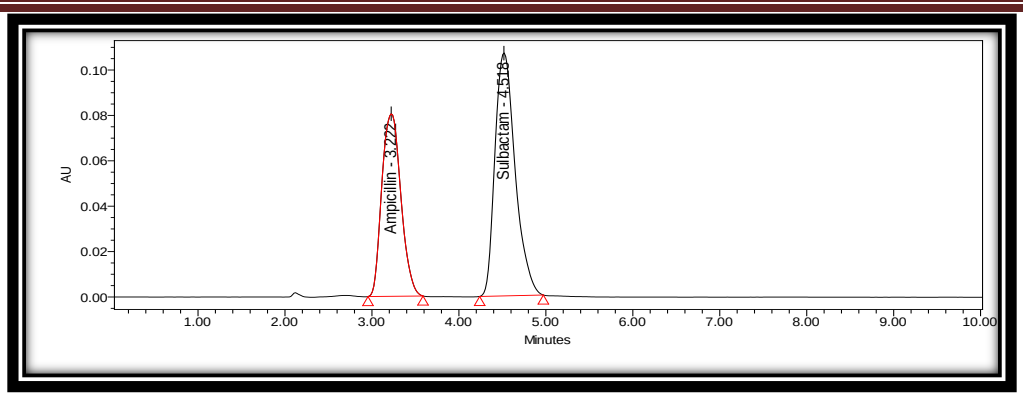

Fig. 6: Chromatograms for Repeatability (standard - 1)

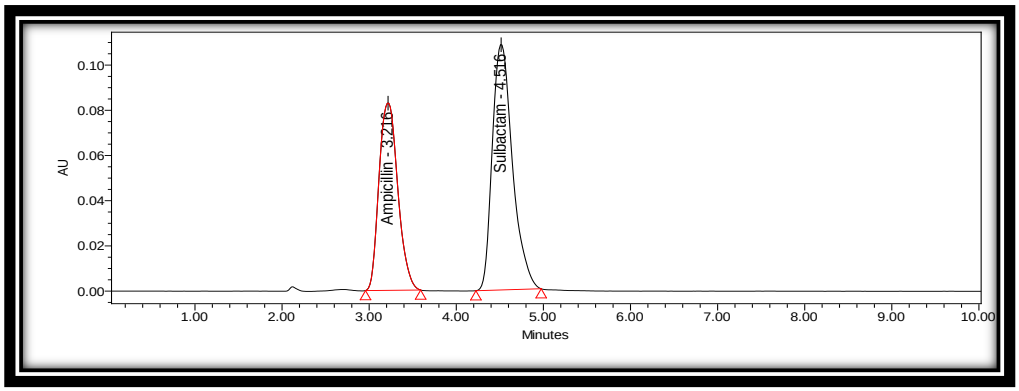

Fig. 7: Chromatogram for Repeatability (standard - 2)

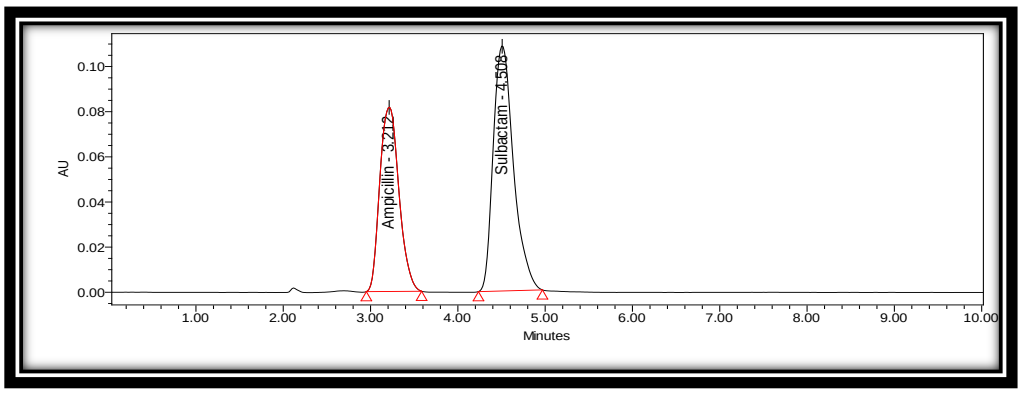

Fig. 8: Chromatogram for Repeatability (standard - 3)

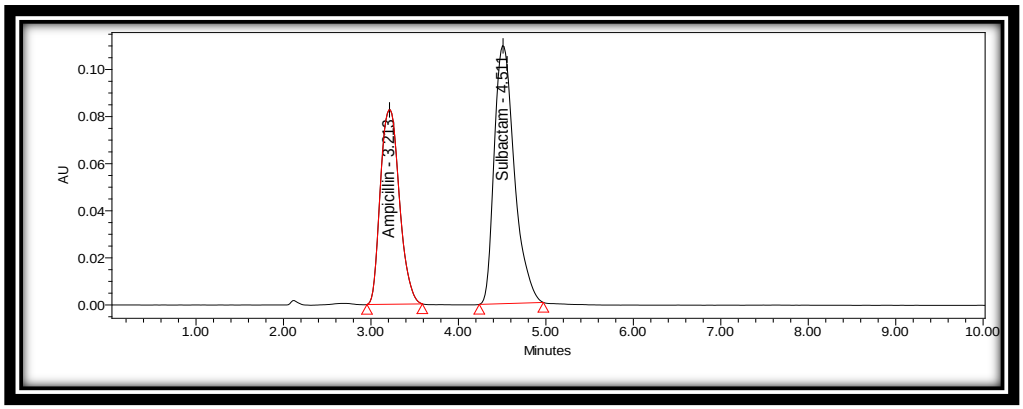

Fig. 9: Chromatogram for Repeatability (standard - 4)

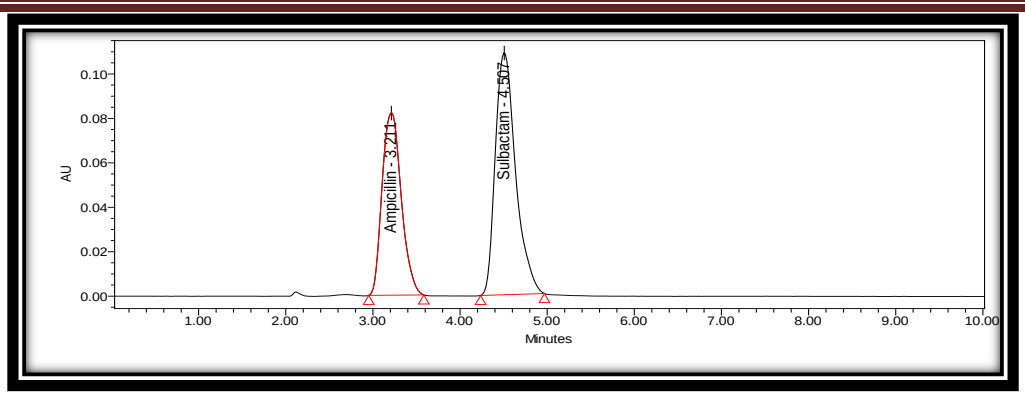

Fig. 10: Chromatogram for Repeatability (standard - 5)

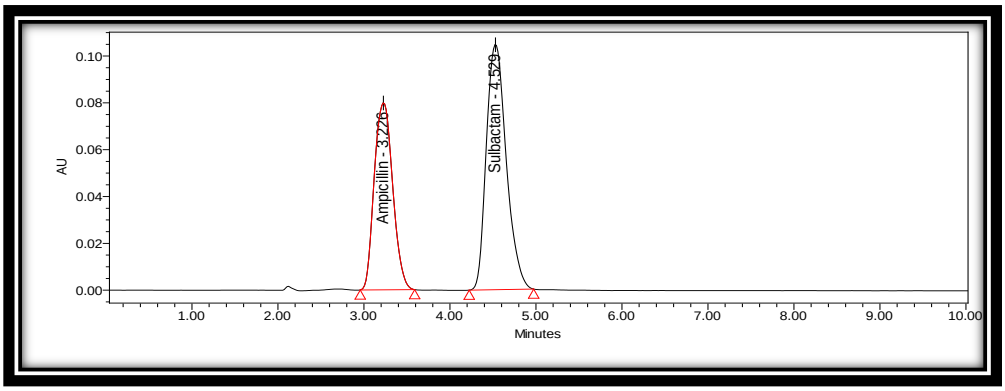

Fig. 11: Chromatogram for Repeatability (standard - 6)

Table No. 5: Results for Intermediate precision

S.No	Peak Area		% Assay	
	Ampicillin	Sulbactum	Ampicillin	Sulbactum
1	2278639	4219201	99.99	99.78
2	224732	4237216	99.66	99.95
3	2267407	4235847	101.53	100.00
4	2254490	4195611	99.98	98.55
5	2231236	4226557	99.97	101.50
6	2267407	4237216	101.17	101.37
% RSD	0.72	0.38	0.75	1.09

Table No. 6: Results for Accuracy of Ampicillin

S No.	Concentration (%)	Original level (µg/ml)	Amount added (µg/ml)	% Recovery	Mean % Recovery	%RSD
1	50	20	20.15	100.75		
2	50	20	19.86	99.31	99.69	0.92
3	50	20	19.80	99.02		
4	100	40	39.88	99.70		
5	100	40	40.12	100.30	99.83	0.41
6	100	40	39.80	99.50		
7	150	60	60.12	100.21		
8	150	60	59.76	99.61	99.97	0.31
9	150	60	60.06	100.10		

Table No. 7: Results for Accuracy of Sulbactum

S No.	Concentration (%)	Original level (µg/ml)	Amount added (µg/ml)	% Recovery	Mean % Recovery	%RSD
1	50	20	20.14	100.32		
2	50	20	19.97	99.85	100.16	0.18
3	50	20	20.02	100.11		
4	100	40	40.01	100.02		
5	100	40	40.05	100.14	100.04	0.10
6	100	40	39.98	99.96		
7	150	60	60.08	100.14		
8	150	60	59.97	99.96	100.2	0.09
9	150	60	59.98	99.98		

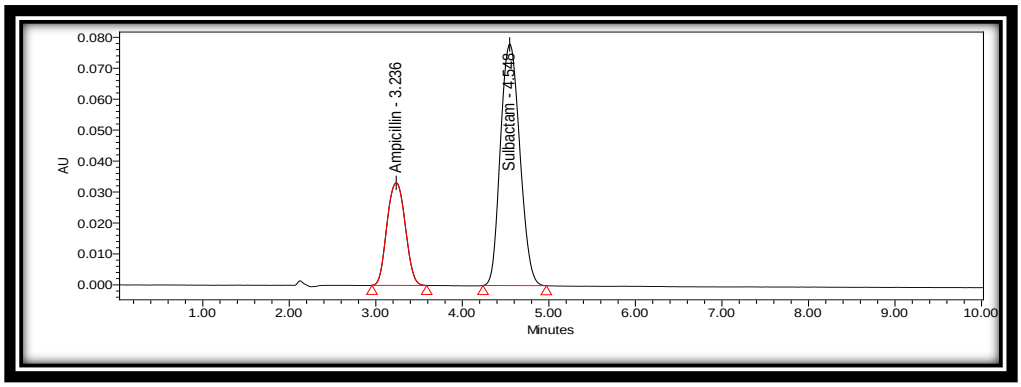

Fig. 12: Chromatogram for accuracy (50%)

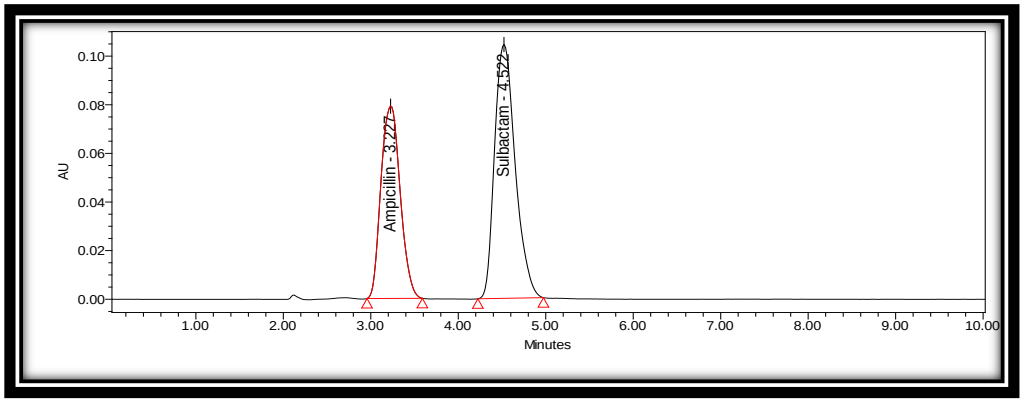

Fig. 13: Chromatogram for accuracy (100%)

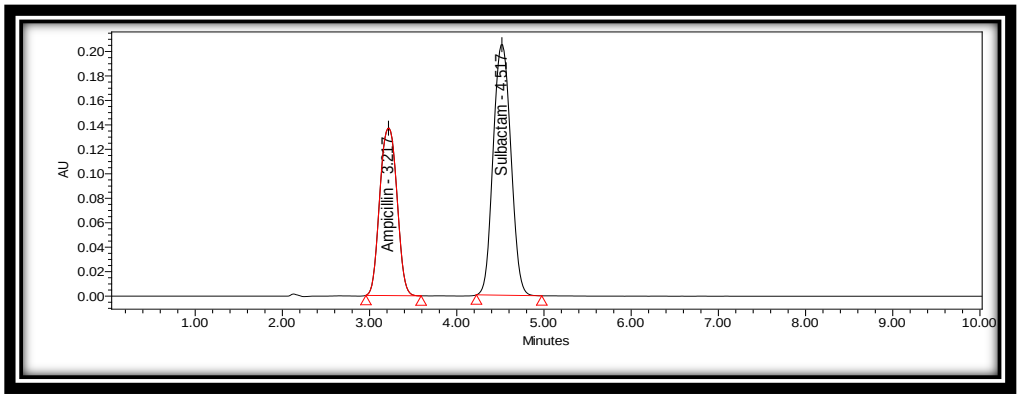

Fig. 14: Chromatogram for accuracy (150%)

Table No. 8: Results for Linearity of Ampicillin and Sulbactam

Analyte	Concentration range (µg/mL)	Correlation Coefficient (R2)	Slope	Intercept
Ampicillin	20-60	0.999	10310	540.63
Sulbactam	20-60	0.999	10893	69769

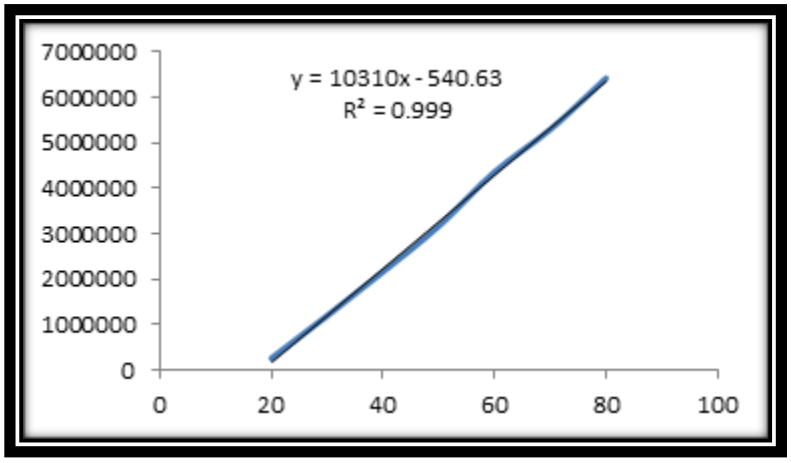

Fig. 15: Linearity Plot for Ampicillin

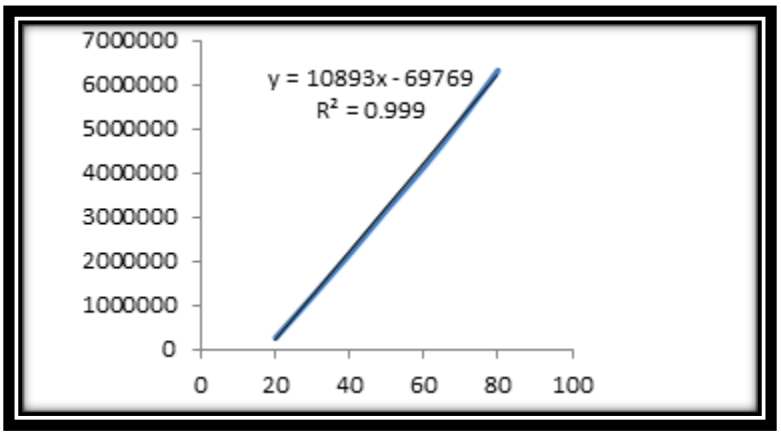

Fig. 16: Linearity Plot for Sulbactam

Table No. 9: Results for Robustness- Ampicillin and Sulbactam

Analyte	Parameters	Adjusted to	Mean Area	Mean RT	%RSD
Ampicillin	Flow Rate ±0.2ml/min	1.0ml/min	2267407	3.222	0.64
		1.4ml/min	2221693	3.021	0.59
	Mobile Phase (Methanol :TBAH	45:55	2135621	2.965	0.86
	(40:60 v/v) (±5ml)	35:65	2215354	3.523	0.56
Sulbactam	Flow Rate ±0.2ml/min	1.0ml/min	4225275	4.754	0.38
		1.4ml/min	4525236	4.231	0.74
	Mobile Phase (Methanol :TBAH	45:55	4128523	4.023	0.62
	(40:60 v/v) (±5ml)	35:65	4125836	4.632	0.65

SUMMARY AND CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Ampicillin and Sulbactum in bulk and Pharmaceutical dosage forms. The method developed has the advantages like required run time, strong separation power.

The advantage of the developed method lies in the simplicity of the stock solution preparation, accurate and less number of reagents was used. And all the validation parameters were within the limits.

It can be concluded that the proposed method can be used for the routine analysis for the Simultaneous estimation of Ampicillin and Sulbactum in bulk and tablet dosage Formby RP-HPLC.

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