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Statistical Comparison, Method Development and Validation of High Performance Liquid Chromatography For Estimation of Sulbactam Sodium and Ampicillin Trihydrate In Bulk and Combined Dosage Form

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ABSTRACT

High performance liquid chromatography for estimation of Sulbactam Sodium and Ampicillin Trihydrate in their combine dosage form was developed and validated. The method was performed on Younglin Instrument with Autochro-3000 Operation software using Varian C-18 (250 X 4.6 mm i.d, 5 µm particle size column and Ammonium acetate Buffer : Acetonitrile: Water (75:17:08, %v/v/v) as mobile phase at ambient temperature. Detection was carried out at 228 nm in the concentration range 25-125 µg/ml for Sulbactam Sodium and 50-250 µg/ml for Ampicillin Trihydrate. The percentage recovery of Sulbactam Sodium and Ampicillin Trihydrate was found to be 99.15 -100.16 and 98.91-103.48 respectively.. Correlation coefficient for Sulbactam Sodium and Ampicillin Trihydrate was found 0.997 and 0.999 respectively. The Rt values for Sulbactam Sodium and Ampicillin Trihydrate were found to be 4.0 min ±0.02 and 5.97 min ±0.03 respectively. The method can successfully applicable to routine analysis. And under Statistical analysis, Paired t-test is applied for comparison between developed method and reported method where, we reject the null hypothesis, because value of t is less than 0.05 so we can conclude that there is significance difference between the developed method and reported method for Sulbactam Sodium and Ampicillin Trihydrate.

Keywords: Sulbactam Sodium, Ampicillin Trihydrate, HPLC, Mobile phase, Column.

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INTRODUCTION

[1,2,3] Sulbactam Sodium is B- lactamase inhibitors and it is chemically(2*S*,5*R*)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide [4,5] Ampicillin Trihydrate is categorized under penicillin antibiotics. and chemically it is (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; trihydrate . Structure of Sulbactam Sodium is illustrated in figure.1. Structure of Ampicillin Trihydrate is illustrated in figure.2.

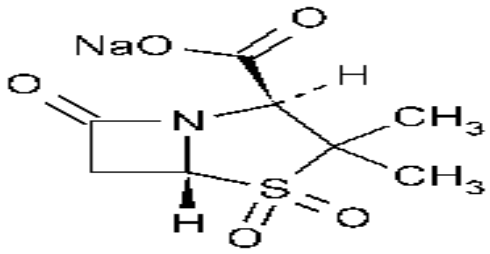


Figure 1: Structure of Sulbactam Sodium

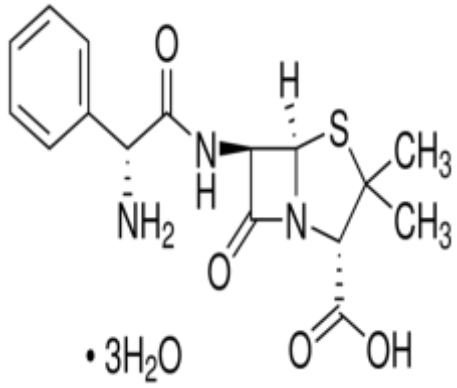


Figure 2: Structure of Ampicillin Trihydrate

Sulbactam Sodium is official in IP[1],BP[2] and USP[3]. Ampicillin Trihydrate is official in BP[4] USP[5]. So far, to our present knowledge, very few HPLC method for estimation of Sulbactam Sodium and Ampicillin Trihydrate has been reported.[6,7] But these methods are time consuming and less sensitive. Hence we focused on developing rapid, Sensitive and cost effective method. The aim of this work is to develop and validate HPLC method[8-12] for the determination of Sulbactam Sodium and Ampicillin Trihydrate in tablet and in active pharmaceutical ingredients and its stastical comparison with previously reported method.

MATERIALS AND METHOD

Detail of Instruments:

Detail of instruments used for study is illustrated in table 1.

Table 1: Detail of HPLC instrument.

Component	Brand/Model/software	Manufacturer/ Supplier
HPLC	Younglin	--
Operation software	Autochro-3000 Operation software	--
Column	Varian C-18 (250 X 4.6 mm i.d, 5 µm particle size	--
Injector	Microliter syringe Injector (Rheodyne)	--
Detector	Dual wavelength UV Detector	--
Filter	Ultipore N_{66} Nylon 6,6 Filter membrane, (0.45µ and 0.2µ)	pall Life sciences

Reagent and chemicals:

Sulbactam Sodium and Ampicillin Trihydrate was a gift sample from Baroque pharmaceutical Limited, Khambhat. All chemicals and reagent used were analytical grade and purchased from Ranbaxy fine chemicals Limited. Combined tablet formulations (Simon) were procured from Indian market.

Preparation of mobile phase:

The mobile phase Ammonium acetate Buffer: Acetonitrile: Water (75:17:08, %v/v/v) respectively was used. The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication.

Preparation of Ammonium Acetate buffer:

An accurately weighed quantity of 0.081 gm ammonium acetate was transferred to 1000 ml volumetric flask and dissolved in 900 ml triple distilled water and volume was made up to 1000 ml with triple distilled water.

Preparation of standard stock solution:

An accurately weighed quantity of Sulbactam Sodium (50 mg) and Ampicillin Trihydrate (100 mg) was dissolved in mobile phase i.e., Ammonium acetate Buffer: Acetonitrile: Water (75:17:08, %v/v/v) in 100 ml volumetric flask and volume was made up to mark with the same solvent. Final strength of solution was 500 µg/ml Sulbactam Sodium & 1000 µg/ml Ampicillin Trihydrate

Preparation of Sample solution:

Weigh and powder 20 tablets. Accurately weighed quantity equivalent to 500 mg of Sulbactam Sodium and 1000 mg of Ampicillin Trihydrate was transferred into 100 ml volumetric flask and dissolved in mobile phase with vigorous shaking. The solution was sonicated for 20 minutes and the volume was made up to mark with the same solvent. The solution was filtered through whatman filter paper No. 41. 1 ml of filtrate was transferred in to 10 ml volumetric flask and

volume was made up to mark with methanol to get the concentration of 500 µg/ml Sulbactam Sodium & 1000 µg/ml Ampicillin Trihydrate. 1.5 ml filtrate from above solution in 10 ml volumetric flask and diluted up to 10 ml with mobile phase to get the concentration of 75 µg/ml Sulbactam Sodium & 150 µg/ml Ampicillin Trihydrate.

Optimized chromatographic conditions:

Optimized chromatographic conditions are illustrated in table 2.

Table 2: Optimized chromatographic conditions

Sr No	Parameter	Chromatographic conditions
1	Stationary Phase	Varian C-18 (250 X 4.6 mm i.d, 5 µm particle size)
2	Mobile Phase	Methanol: water: Potassium dihydrogen phosphaste Buffer (pH 3) (30:05:65,
3	Mode of Elution	Isocratic
4	Temperature	25ºC
5	Flow rate	1 ml/min
6	Injection Volume	10 µl
7	Wavelength of detection	228 nm

RESULT AND DISCUSSION[11-18]

The method was validated by establishing linearity, accuracy, interday and intraday precision of measurement of sample application. The limit of detection and limit of quantification were also determined. Chromatogram Sulbactam Sodium and Ampicillin Trihydrate in standard solution is shown in figure no 3. Chromatogram of Sulbactam Sodium and Ampicillin Trihydrate in active pharmaceutical ingredients is shown in figure 4, 5. Chromatogram of Sulbactam Sodium & Ampicillin Trihydrate in sample solution is shown in figure 6.

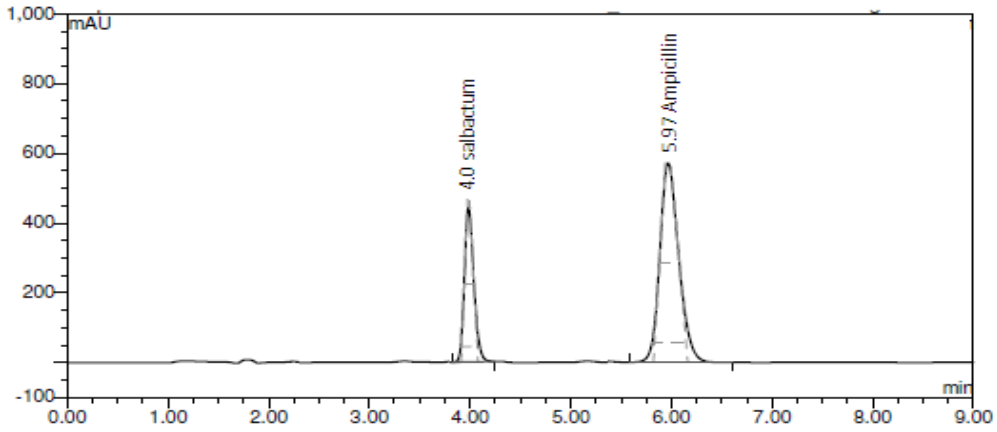


Figure 3: Chromatogram of Sulbactam Sodium & Ampicillin Trihydrate in standard solution.

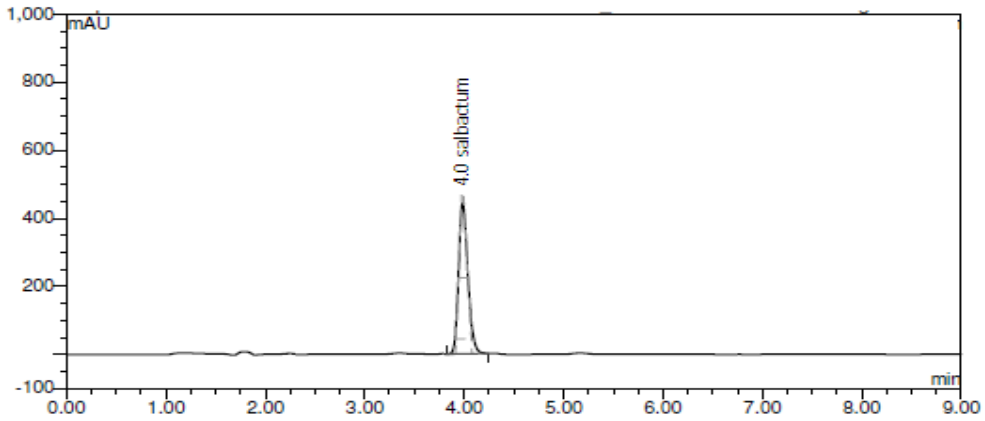
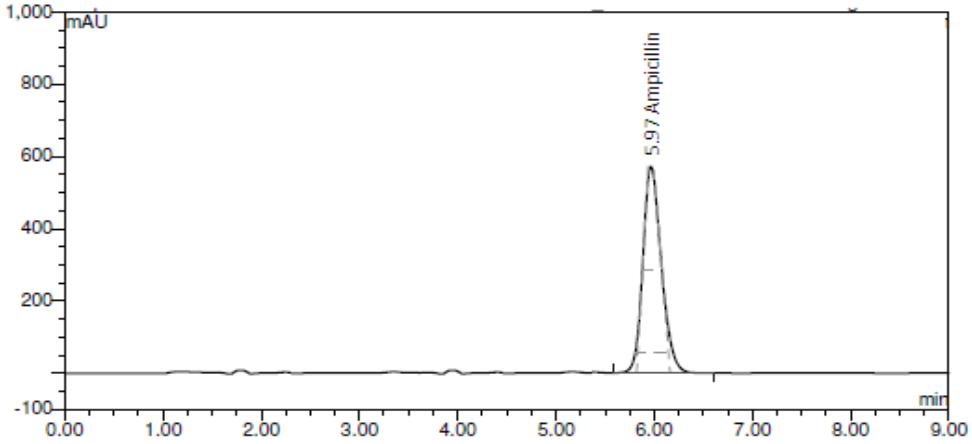


Figure 4: Chromatogram of Sulbactum Sodium in standard solution



Linearity calibration curve: [11-12]

A calibration curves were plotted over a concentration range of 25-125 µg/ml for Sulbactam Sodium and 50-250 µg/ml for Ampicillin Trihydrate. Accurately measured standard stock solutions of Sulbactam Sodium & Ampicillin Trihydrate (5,10,15,20 and 25 ml) were transferred to a series of 10 ml volumetric flasks and the volume in each flask was adjusted to 10 ml with mobile phase. The resulting solution was injected into the column with flow rate 1 ml/min were measured at 228 nm for Sulbactam Sodium & Ampicillin Trihydrate. Calibration curves were constructed for Sulbactam Sodium & Ampicillin Trihydrate by plotting peak area versus concentration at 228 nm. Five concentration points were assayed in triplicate. Both Sulbactam Sodium & Ampicillin Trihydrate showed good linearity in tested range. The regression coefficient (R2) Value for Sulbactam Sodium & Ampicillin Trihydrate were found to be 0.997 and 0.999 respectively. Linear regression data for the calibration plots (n=6) are illustrated in figure no 7 and 8 respectively.

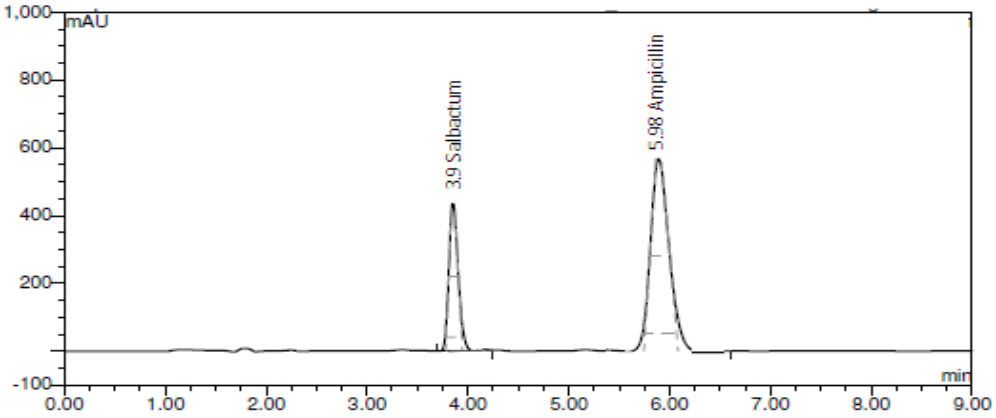


Figure 6: Chromatogram of Sulbactam Sodium & Ampicillin Trihydrate in sample solution.

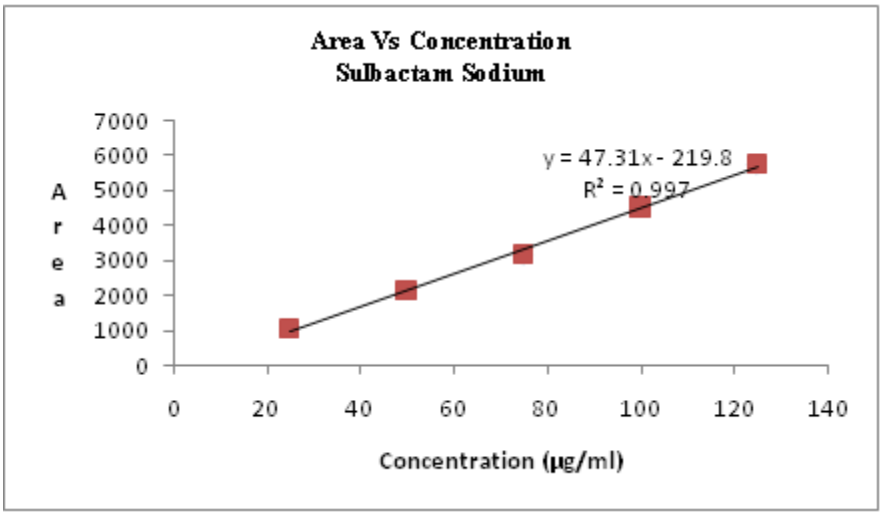


Figure 7: Calibration graph of Sulbactam Sodium.

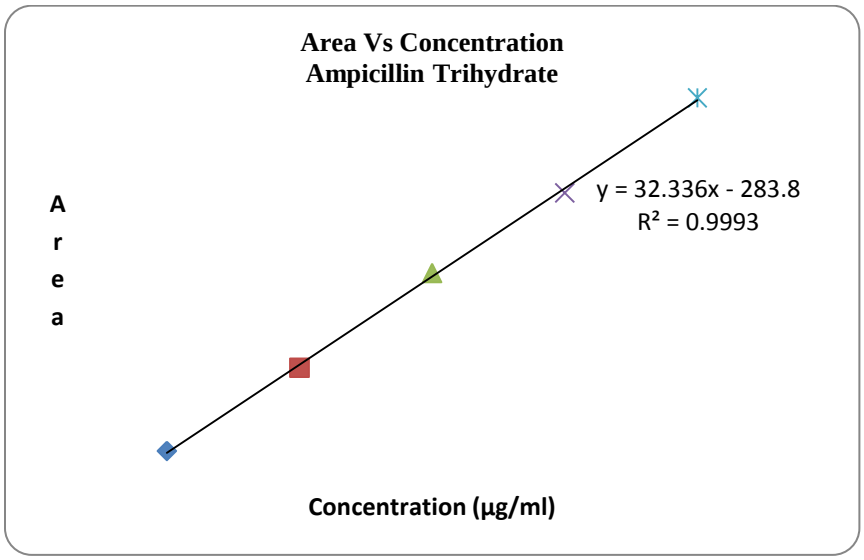


Figure 8: Calibration graph for Ampicillin Trihydrate

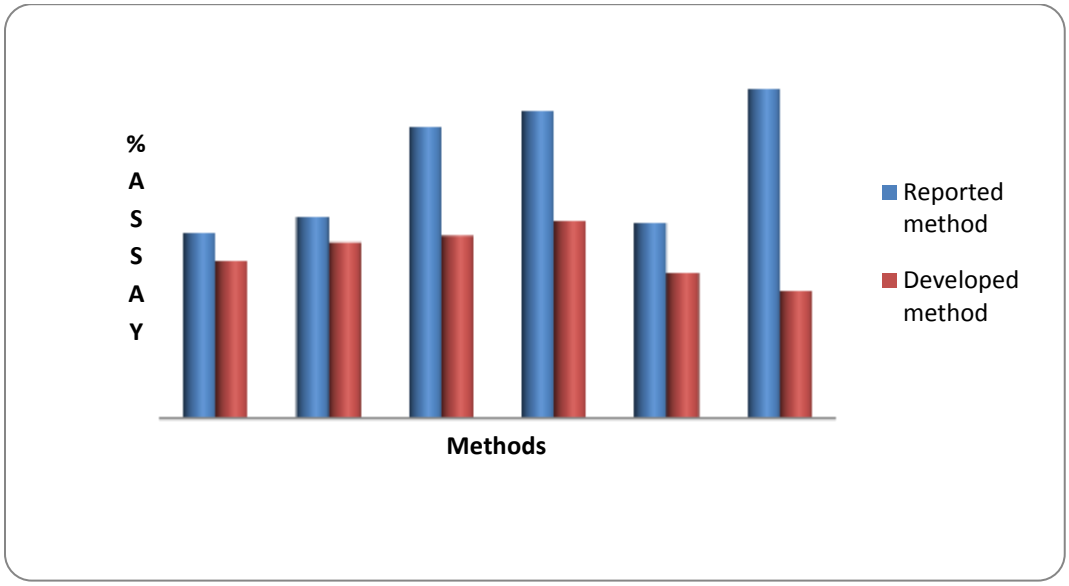


Figure 9: Statistical Comparison between reported method and developed method for Sulbactum Sodium.

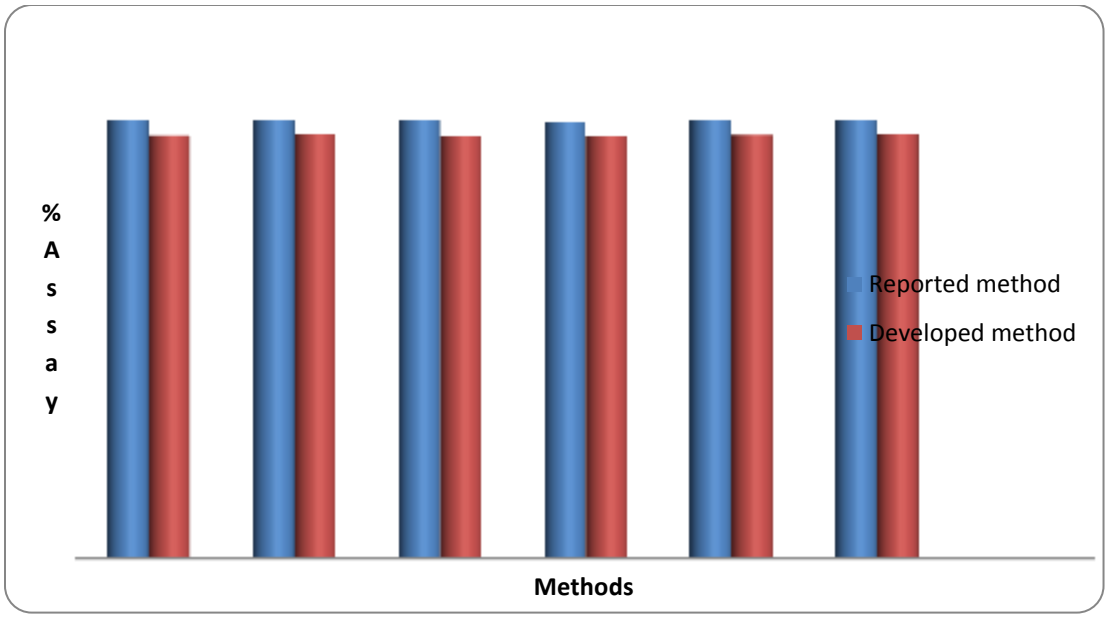


Figure 10: Statistical Comparison between reported method and developed method for Ampicillin Trihydrate.

Accuracy:

Accuracy may often be expressed as % Recovery by the assay of known, added amount of analyte. It is measure of the exactness of the analytical method. The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the tablets (Sulbactam Sodium 75 µg/ml and Ampicillin Trihydrate 150 µg/ml) with three different concentrations of standards at 80 %, 100 % and 120 % Sulbactam Sodium (60,75,90 µg/ml) and Ampicillin Trihydrate

(120,150,180 µg/ml). The % recovery and %RSD were calculated and found to be within the limit as shown in table 3.

Table 3: Recovery data for Cefixime Trihydrate and Potassium Clavunate

Level	Concentration (µg/ml)		Spike solution		Area of spike solution		% Assay	
	Sul	Ampi	Sul	Ampi	Sul	Ampi	Sul	Ampi
80%	75	150	60	120	3012	4213	99.15	101.28
100%	75	150	75	150	3712	5103	99.05	99.38
120%	75	150	90	180	4492	6192	100.86	101.53

*= Average result of three replicate samples

Precision: [11-12]

Intraday precision was found by analysis of standard drug at six times on the same day. While interday assay precision was carried out on six different day. The RSD was found to be less than 2 for both interday precision and intraday precision. Result for the interday precision and intraday precision is shown in table 4 .

Table 4: Data of Interday Precision and Intraday precision

Concentration(µg/ml)		Area		% Assay		%RSD	
Sul	Ampi	Sul	Ampi	Sul	Ampi	Sul	Ampi
Interday Precision							
75	150	3704	5108	98.83	99.48	0.258	0.322
Intraday precision							
75	150	3715	5134	99.14	100.01	0.386	0.332

*= Average result of six replicate samples

LOD and LOQ: [11-18]

Limit of Detection and Limit of quantitation are calculated based on calibration Curve and the results are shown in table 5.

Table 5: Results of LOD and LOQ.

Parameter	Compound	Result
Detection limit	Sulbactam Sodium	0.633 µg/ml
	Ampicillin Trihydrate	1.587 µg/ml
Quantitation limit	Sulbactam Sodium	1.917 µg/ml
	Ampicillin Trihydrate	4.810 µg/ml

Robustness: [11-12]

Robustness data clearly shows that the proposed method is robust at small but deliberate change. Results of robustness are shown in table 6.

Table 6: Data of robustness

Chromatographic factor	level	Retention time		Tailing factor	
		Sulbactam Sodium	Ampicillin Trihydrate	Sulbactam Sodium	Ampicillin Trihydrate
Flow rate	0.8ml/min	4.01±0.03	5.93±0.02	0.98±0.02	0.97±0.01
	0.5ml/min	4.07±0.01	5.97±0.05	0.97±0.05	0.96±0.03
Ammonium acetate	73:16:11, %v/v/v)	4.03±0.01	5.98±0.04	0.97±0.03	0.96±0.08
Buffer : Acetonitrile: Water (75:17:08, %v/v/v)	76:15:09, %v/v/v)	4.06±0.03	5.95±0.06	0.97±0.04	0.97±0.08
Detection wavelength	225nm	4.04±0.05	5.94±0.02	0.98±0.02	0.97±0.01
	230nm	4.06±0.02	5.93±0.01	0.97±0.01	0.97±0.02

Application of proposed method to the Pharmaceutical dosage form:

The proposed method was applied successfully to the tablet dosage form and results obtained are shown in table 7.

Table 7: Application of the method to the determination of tablet dosage form.

Formulation	Sulbactam Sodium			Ampicillin Trihydrate		
	Amount taken (µg/ml)	Amount found (µg/ml)	% Amount found (n=3)	Amount taken (µg/ml)	Amount found (µg/ml)	% Amount found (n=3)
Simon	500	495.56	99.12	1000	999.31	99.93

*= Average result of six replicate samples

System Suitability Parameters[11-12]

Statistical analysis of parameters required for system suitability testing of the HPLC method. Data related to system suitability parameters are shown in 8.

Table 8: Data of system suitability parameters.

Parameter	RT*	AUC*	No. of theoretical plates*	Tailing factor*
Sulbactam Sodium	4.0±0.07	226752.6±4003.35	4390.4±90.52	0.97±0.02
Ampicillin Trihydrate	5.97±0.03	273205.2±312.53	3237.4±139.80	1.08±0.01

*=Average result of six replicate samples

Table 9: Summary of validation parameter

Parameter	Sulbactam Sodium	Ampicillin Trihydrate
Wavelength	228 nm	228 nm
Range	25-125 µg/ml	50-250 µg/ml
Linearity	0.997	0.999
%RSD (Interday pricision)	0.258	0.322
%RSD (Intraday pricision)	0.384	0.332
LOD	0.633 µg/ml	1.587 µg/ml
LOQ	1.917 µg/ml	4.810 µg/ml

Statistical Comparison between reported method and developed method [6-7],[19]:

Application of Paired t-test:

For Sulbactum Sodium

t-Test: Paired Two Sample for Means		
	Reported method	*Developed method*
Mean	99.42666667	99.14166667
Variance	0.045026667	0.007136667
Observations	6	6
Pearson Correlation	0.074008093	
Hypothesized Mean Difference	0	
df	5	
t Stat	3.137429835	
P(T<=t) one-tail	0.012870409	
t Critical one-tail	2.015048372	
P(T<=t) two-tail	0.025740818	
t Critical two-tail	2.570581835	

Interpretation: p value 0.012870409 < 0.05 so, we reject the null hypothesis, and conclude that there is significance difference between the all developed methods for Sulbactum Sodium

t-Test: Paired Two Sample for Means		
	Reported method	*Developed method*
Mean	103.4766667	99.95166667
Variance	0.034546667	0.005016667
Observations	6	6
Pearson Correlation	0.444117245	
Hypothesized Mean Difference	0	
df	5	
t Stat	51.72092247	
P(T<=t) one-tail	2.5539E-08	
t Critical one-tail	2.015048372	
P(T<=t) two-tail	5.10781E-08	
t Critical two-tail	2.570581835	

Interpretation: p value 2.5539E-08 < 0.05 so, we reject the null hypothesis, and conclude that there is significance difference between the all developed methods for Ampicillin Trihydrate.

Graphical representation of Comparison between reported method and developed method is shown in figure no 9 and 10 for Sulbactam Sodium and Ampicillin Trihydrate respectively.

DISCUSSION:

The results indicated that the proposed method was simple, sensitive, accurate and precise for simultaneous estimation of Sulbactam Sodium and Ampicillin Trihydrate in tablet dosage form by HPLC.

Separation of drugs was carried out using Ammonium acetate Buffer : Acetonitrile: Water (75:17:08, % v/v/v) mobile phase with wavelength 228 nm. The Rt values for Sulbactam Sodium and Ampicillin Trihydrate were found to be 4.0 ±0.05 and 5.97±0.03 respectively.

The drug response with respect to peak area was linear over the concentration range 25-125 µg/ml for Sulbactam Sodium and 50-250 µg/ml for Ampicillin Trihydrate. The percentage recovery of Sulbactam Sodium and Ampicillin Trihydrate was found to be 99.05-100.86 and 98.06-101.53 respectively.

The % RSD values for intraday precision and interday precision study were ≤2.0% conforming that the method was sufficiently precise. The limit of detection and limit of quantification were found to be 0.633 µg/ml and 1.917 µg/ml for Sulbactam Sodium and for Ampicillin Trihydrate 1.587 µg/ml and 4.810 µg/ml.

The %RSD values of robustness study were ≤2.0%, conforming that the proposed method was found to be robust enough to withstand such deliberate changes and allow routine analysis of sample.

The system suitability parameters were also performed and were found within acceptable range.

This method can be successfully employed for simultaneous estimation of Sulbactam Sodium and Ampicillin Trihydrate in bulk and dosage form.

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