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STUDIES ON ANALYSIS AND STABILITY OF SULTAMICILLIN AND ITS TABLET FORMULATION

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ABSTRACT

Ampicillin is a β-lactam antibiotic that is advantageously combined with sulbactam, β-lactmase inhibitor to extend antimicrobial spectrum of Ampicillin. The combination is official in USP as injectable preparation. The combination cannot be administered as orally as sulbactam is not stable at an acidic pH. Sultamicillin is a novel compound that helps to ensure administration by oral route. Sultamicillin is mutual prodrug formed by reacting Ampicillin and Sulbactam with methylene diol to form diester with carboxylic acid group of both the drugs. Sultamicillin hydrolyses into Ampicillin and Sulbactam into body, the desired drugs. Sultamicillin is difficult to handle and not very stable in solid form. Sultamicillin Hydrochloride salt greatly enhances solubility but does not solve the problem of handling difficulty and stability in solid state. Salt with toluene sulfonic acid called sultamicillin tosilate solves the problem and this is official in European Pharmacopeia and British Pharmacopeia. Sultamicillin is marketed as 375 mg capsules world over and in India for oral use. The capsules are indicated for various systemic infections including anerobes and are also used empirically where causative organisms are not known. The combination is currently used in special indications of antimicrobial therapy. Sultamicillin is an oral form of mutual prodrug antibiotic containing ampicillin, a β-lactam antibiotic and sulbactam, a β-lactamase inhibitor. Sultamicillin is used orally as tablets and is as effective as ampicillin and sulbactam injection in the empirical therapy of variety of infections. The objective was to develop suitable UV spectrophotometric and HPLC method that can separate Sultamicillin from its hydrolysis products- ampicillin and sulbactam so that they can be used for thermal & hydrolytic stability study of the drug and its tablet dosage form.

KEYWORDS: Ampicillin, Sulbactam, Sultamicillin, Sultamicillin tosilate, Toluene sulphonic acid, Mutual prodrug, Solubility, logP, λ_{max}-UV, IR, Reversed phase HPLC, Student's t-test.

OBJECTIVE: The objective was to develop UV spectrophotometric and simple HPLC method that can separate sultamicillin from ampicillin and sulbactam so that can be used for stability study of the drug and its tablet dosage form.[1,2]

INTRODUCTION

There are no patents or research publications showing data on stability of Sultamicillin as bulk drug or dosage form despite its instability. The constituent drugs Ampicillin and Sulbactam formed by hydrolysis of the drug are in fact estimated as impurities in Sultamicillin capsules. It was therefore thought proper to develop suitable stability indicating methods and study stability of the drug under hydrolytic conditions.[3,4]

Sultamicillin (CAS Registry Number: 76497-13-7)

Figure-1: Sultamicillin tablet & powder (API).

Supplier: Sultamicillin: Aurobindo Pharma Limited, Hyderabad; Sultamicillin tablets (Saltum: 375mg), Morepen. Sultamicillin is an oral form of the antibiotic made of ampicillin and sulbactam. It contains esterified ampicillin and sulbactam and is marketed. The pharmacokinetic properties of sultamicillin are improved compared to a combination. Sultamicillin increases the absorption and decreases the chances of diarrhea and dysentery. The inclusion of sulbactam extends ampicillin's spectrum of action to β-lactamase producing strains of bacteria. Oral sulbactam with parenteral form provides a regimen of continuous sulbactam therapy throughout the treatment, resulting in better clinical results.[5,6]

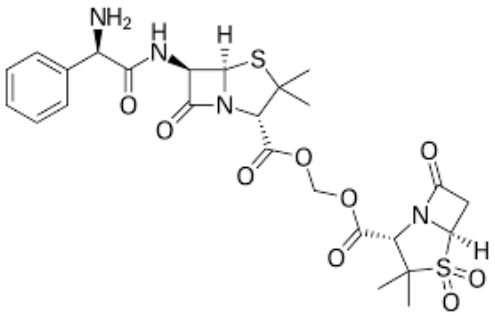


Figure-2: Sultamicillin.

Chemistry: (2R)-3,3-Dimethyl-4,4,7-trioxy-4λ6-thia-1-azabicyclo[3.2.0]heptane-2-carbonyl]oxymethyl(2R)-6-{[(2S)-2-amino-2-phenyl-acetyl]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylate.

It is an azabicyclo[3.2.0]heptane penam ring. It has six chiral points out of that 2R is common in which ampicillin unit and sulbactam units are joined by ester linkages with methylene diol, amino part of ampicillin is connected by 2S linkage, 6th position of ampicillin is connected with imino nitrogen of thia-1-azabicyclo[3.2.0]heptane, so it is 4λ6-thia. It has molecular weight=594.659g/mol, molecular formula=$C_{25}H_{30}N_4O_9S_2$, logP=1.55, pKa=11.71 (acidic property generates from keto-enol tautomerism of amide linkage [-CONH-] of the chain & 7.23 (basic property generates from primary amino of chain and tertiary nitrogen atoms of the two rings), water solubility=0.283mg/mL, melting point=190°C. It is highly nonpolar so it's water solubility is 0.283mg/mL and logP is 1.55.[7,8]

Sultamicillin is official drug in European Pharmacopoeia. Sultamicillin [$C_{25}H_{30}N_4O_9S_2$] is a mutual prodrug of sulbactam and ampicillin attached with methane. Sultamicillin hydrolyses into sulbactam and ampicillin by cleavage of ester made by covalent bond by hydrolysis. Sultamicillin is a prodrug of ampicillin and β-lactamase inhibitor sulbactam, it consists of two compounds linked as a double ester.

During absorption from gastrointestinal tract it is hydrolyzed, releasing equimolar quantities of sulbactam and ampicillin. Sultamicillin is given orally as tablets containing sultamicillin tosilate or as oral suspension containing sultamicillin. It is used in the treatment of infections where β-lactamase producing organisms might occur, including uncomplicated gonorrhea, otitis media, respiratory tract and urinary tract infections. The usual dose is 375-750mg of sultamicillin equivalent to 147-294mg of sulbactam and 220-440mg of ampicillin. Ampicillin, a semi-synthetic orally active broad spectrum antibiotic, is linked via a methylene group with a β-lactamase inhibitor. Sultamicillin is chemically oxymethyl penicillinate sulfone ester of ampicillin.[9-11]

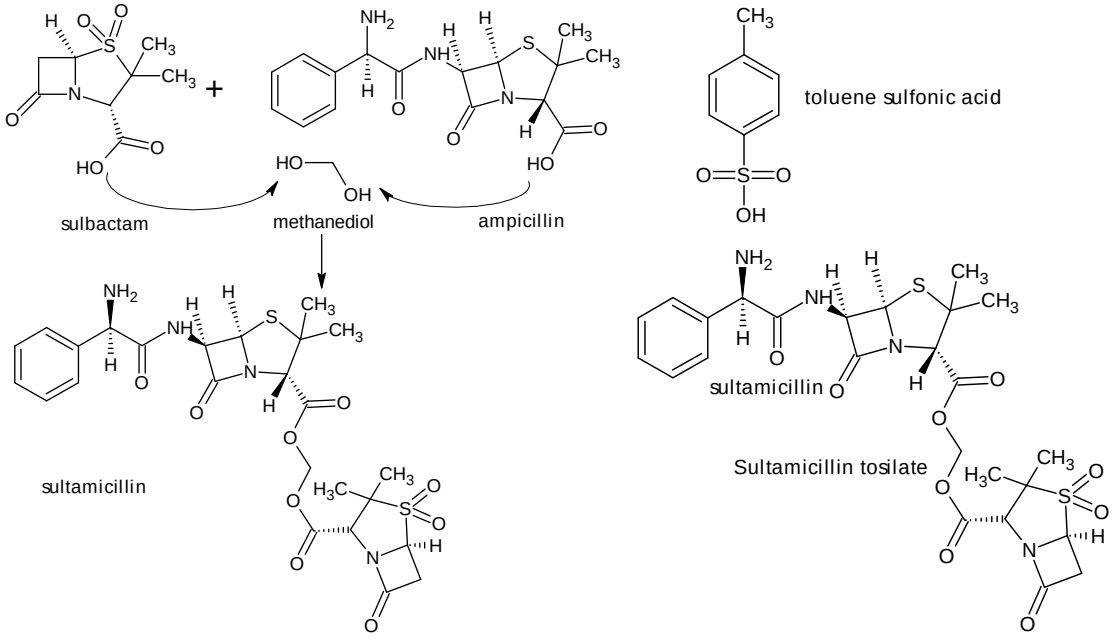


Figure-3: Sultamicillin & Sultamicillin tosilate prodrug.

After absorption, sultamicillin releases ampicillin and sulbactam into the system, so all the antibacterial efficacy of sultamicillin is due to ampicillin and sulbactam. Ampicillin exerts antibacterial activity against sensitive organisms by inhibiting biosynthesis of cell wall mucopeptide where as sulbactam irreversibly inhibits most important β-lactamases that occur in resistant strains. It is made by esterfication of sulbactam and ampicillin with methylene diol [$CH_2(OH)_2$] where one –COOH of sulbactam and one –COOH of ampicillin esterfies with two –OH of methylene diol to produce the desired mutual prodrug.[12]

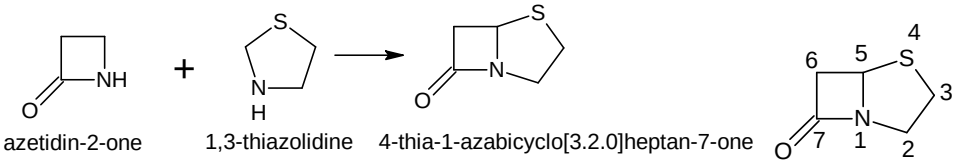


Figure-4: Carbopenam fused ring system.

All three moieties have same 4-thia-1-azabicyclo[3.2.0]heptan-7-one fused ring which is formed by fusion of azetidin-2-one with 1,3-thiazolidine. In heterocyclic ring system counting starts from Oxa (O)/Thia (S)/Aza (N) but in bicyclic fused ring nomenclature counting is reversed Aza (N)/Thia (S)/Oxa (O), so here nitrogen comes first (1-aza) in counting and for this sulphur is in 4th number (4-thia), so from right hand side after nitrogen 3 is for sulphur and from left hand side after sulphur nitrogen comes at 2 and both azetidin-2-one and 1,3-thiazolidine fuses at 0 so [3.2.0] is coming and 7 number comes from counting from nitrogen so heptan is coming. 4-thia-1-azabicyclo[3.2.0]heptan-7-one ring system comes then with 7th position is ketone (7-one).[13,14]

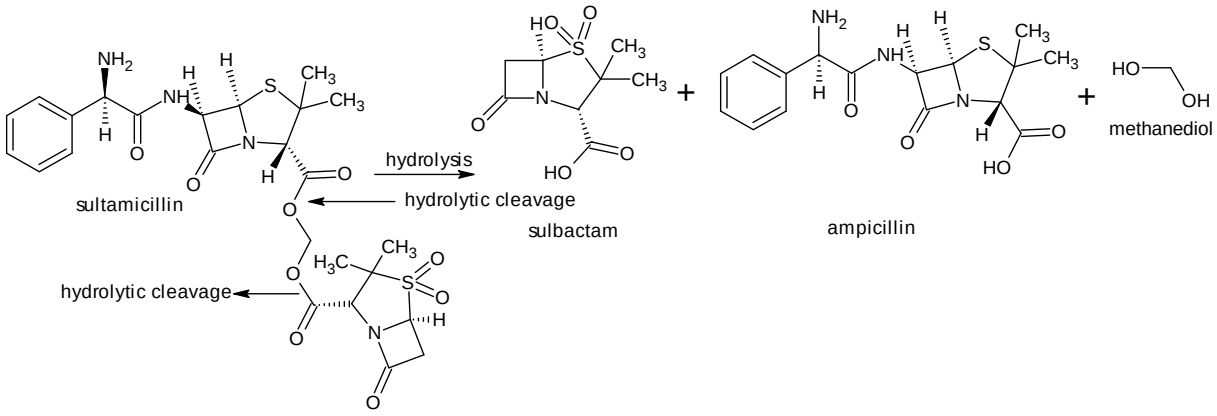


Figure-5: Sultamicillin hydrolyses in physiological fluid into it's two components: sulbactam & ampicillin.

Experimental Methods: Simple UV spectrophotomertic method was developed for estimation of sultamicillin in tablet dosage form by measuring absorbance at maximum at 278 nm. The method exhibited good linearity from 10-100 µg/ml and was used at 50 µg/ml concentration for assay. The method has been validated as per ICH guidelines.

RP-HPLC test method was developed with a view to separate sultamicillin from ampicillin and sulbactam, the likely hydrolysis products with partial success. The sulbactam could be adequately separated from immediately preceding peak mostly believed to be due to tosilate. The developed method could satisfactorily separate sultamicillin from sulbactam but not from ampicillin. Of the four types of columns tried C8, C18, Hilic and Phenylhexyl, the Phenonenex make phenylhexyl column was found to be more suitable in achieving the separation. The method exhibited good linearity from 200-1000 µg/mL and was used at 100 µg/ml concentration for assay. The method has been validated as per ICH guidelines.

It is white crystalline powder insoluble in water and sparingly soluble in methanol. It has six chiral centres so 2^6= 64 enantiomers are possible. Specific rotation: +168 to +186º (C=0.5, water/CH_3CN=3:2), Melting Point=190ºC.[15,16]

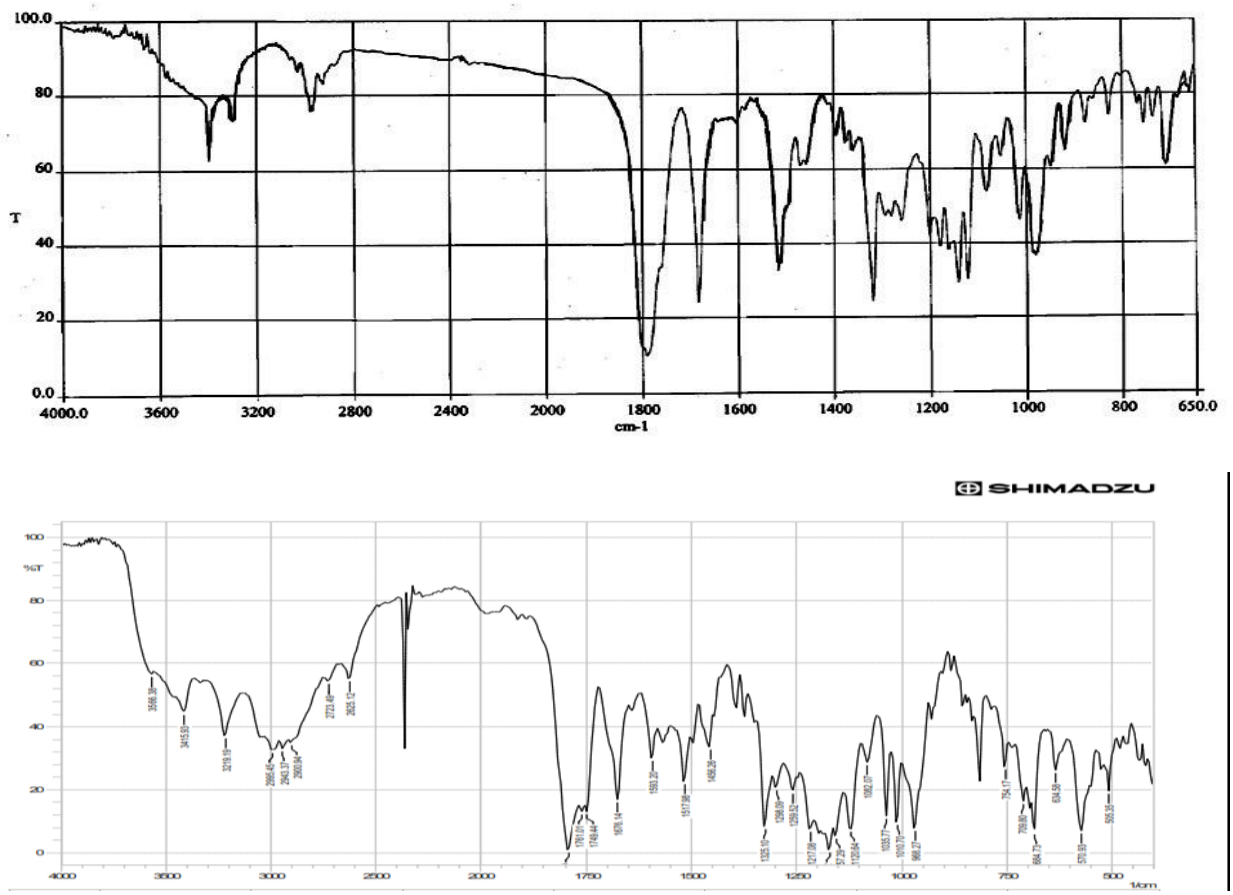


Figure-6: Infra Red spectra of Sultamicillin tosilate (Standard & API).

IR spectrum: υ (cm^{-1}): CH_3 (Reported: 2970-2950; Observed: 2943.3), CH_2 (Reported: 2935-2915; Observed: 2900.94), C_6H_5 (Reported: 1510-1450; Observed: 1517.98), NH_2 (Reported: 3510-3460; Observed: 3566), -CONH- (Reported: 1680-1630; Observed: 1676.14), -COO- (Reported: 1750-1725; Observed: 1749.44), O=S=O (Reported: 1365-1340; Observed: 1325), -CO- (Reported: 1725-1705; Observed: 1749.44), $-SO_3H$ (Reported: 1100–1200; Observed: 1217.06)

UV Spectrophotometric Method estimation of sultamicillin tosilate:

➢ **Instrument:** A double beam UV Visible Spectrophotometer

- Manufacturer :Shimadzu
- Model :UV-1800, Shimadzu, Japan with UV Probe 2.31

➢ **Preparation of standard solution:**

About 10mg of Sultamicillin tosilate was weight and dissolved in 100ml volume flask with methanol. This make 100µg/ml concentration, The UV Spectrum of the solution was recorded from 200-400 nm using spectrum modes. The spectrum is recorded.[17,18]

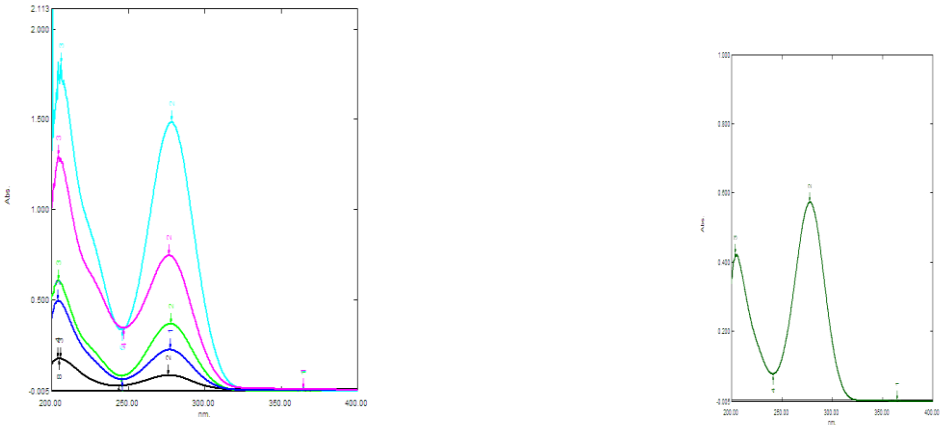


Figure-7: UV Maximum Absorbance λmax of Sultamicillin.

➢ **Preparation of calibration curve:**

Aliquot of 0.1, 0.2, 0.4, 0.6, 0.8 and 1ml of stock solution (100µg/ml) where pipette in 10ml volumetric flask. Methanol was added to volume and mixed. This gave 10, 20, 40, 60, 80 and 100µg/ml concentration. And the spectra of this solution were scanned from 200-400nm using spectrum mode.

- Method for estimation of sultamicillin in to solution.

- **Preparation of test (sample):**

Marketed sample of sultamicillin 375mg tablets (sultam) well taken for analysis. Twenty tablets weight determined (0.602gm) the tablets cursed and quantity of powder equivalent to 100mg (0.160gm) was transferred to 100ml volume flask and about 30ml methanol was added and sonicated for 10 minutes. The volume was made up to mark with methanol and mixed aliquot of 0.5ml was diluted to 10ml with methanol. This gives 50µg/ml concentration. The UV Spectra was recorded using spectrum mode and absorbance was measured at λmax of 278nm.[19,20]

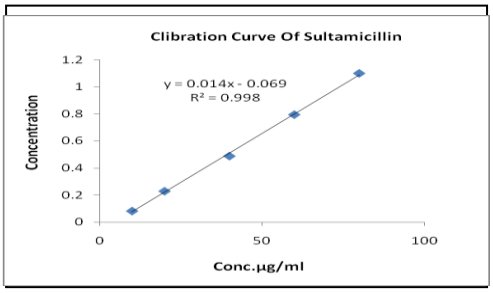
Calculation of Assay:

The amount of sultamicillin tosilate equivalent to sultamicillin in test preparation was calculated from calibration curve standard equation.

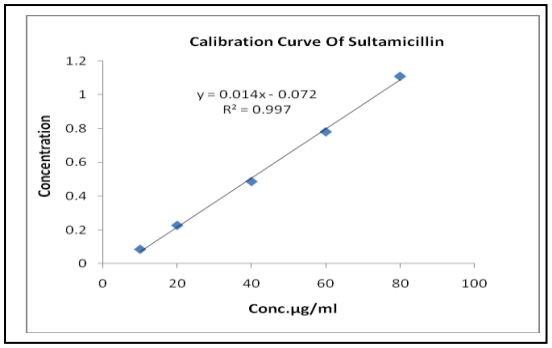
Validation of UV Spectrophotometric Method:

Linearity:

Conc. µg/ml	Concentration
10	0.082
20	0.229
40	0.489
60	0.796
80	1.102
100	1.478



Conc. µg/ml	Concentration
10	0.084
20	0.226
40	0.486
60	0.781
80	1.110



Conc. µg/ml	Concentration
10	0.080
20	0.232
40	0.500
60	0.805
80	1.008

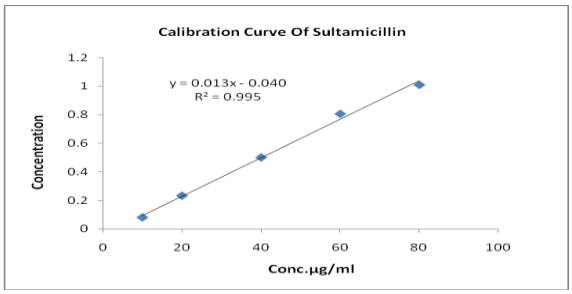


Figure-8: Calibration curve of Sultamicillin.

Table-1: Absorbance of Sultamicillin.

Concentration	Absorption	SD	%RSD
10	0.0813	0.001528	1.87
20	0.2236	0.0025	1.12
40	0.482	0.0036	0.77
60	0.6973	0.0055	0.78
80	1.312	0.01026	0.72

Precision:

1. Repeatability (Intra-day precision)

Intra-day precision was determined by analyzing of sultamicillin solution in the range 10, 20, 40, 60 and 80µg/mL triplicate on the same day. Calculated % RSD for sultamicillin.

2. Inter-day precision (different days)

Intra-day precision was determined by analyzing of sultamicillin solution in the range10, 20, 40, 60 and 80µg/ml in different days. Calculated %RSD for sultamicillin.

Table-2: Intra-day precision data for estimation of sultamicillin.

Conc. (µg/ml)	Mean peak Area±SD (n=3)	%RSD
10	0.082±0.002	2.43
20	0.229±0.003	1.31
40	0.491±0.007	1.49
60	0.794±0.0121	1.52
80	1.102±0.001	0.090

Table-3: Inter–day Precision data for sultamicillin.

Conc. (µg/ml)	Mean peak Area±SD (n=3)	%RSD
10	0.083±0.001	1.20
20	0.225±0.0025	1.11
40	0.486±0.005	1.02
60	0.781±0.0125	1.59
80	1.121±0.001	0.089

Accuracy:

Table-4: Accuracy.

Test Conc.	Std Conc.	Mean Peak area	Amount found	%Recovery	%SD	%RSD
25	15	0.478	39.95	99.87	1.83	1.83
25	25	0.648	49.98	102.69	1.0	0.97
25	35	0.780	59.99	100.0	1.2	1.2

LOD: LOD and LOQ

The limit of detection (LOD) was found to be 1.41µg/ml; while the limit of quantification (LOQ) was found to be 4.29µg/ml for sultamicillin.[21]

=3.3σ/slope

=3.3 (0.07/0.013)

=1.41µg/ml

LOQ:

=10.0σ/slope

=10 (0.007/0.0013)

=4.29µg/ml

Tablet Assay:

Table-5: Tablet Assay of Sultamicillin.

Tablet Formulation	Label claim	%Assay±%RSD
Sultamicillin tablet	375mg	98.34±0.97

RP-HPLC method for estimation of sultamicillin:

Experimental:

a) Reagents and Materials:

- Sultamicillin tosilate EP
- Methanol (HPLC grade, Merck Specialties Private Ltd, Mumbai, India)
- Acetonitrile (HPLC grade, Merck Specialties Private Ltd, Mumbai, India)
- Potassium Dihydrogen Ortho Phosphate
- Ortho phosphoric acid
- Water (HPLC grade, RFCL Limited, New Delhi, India).

b) Equipments and Instruments:

- Shimadzu HPLC instrument (LC-2010 CHT)(software LC Solution)
- Analytical balance (Acculab ALC-210.4, Huntingdon Valley, PA)
- Ultra sonicator (EN 30 US, Enertech Fast Clean, Mumbai, India)
- Hot air oven (TO-90S, Thermo lab)
- pH meter (Thermo Electron Crop., Pune, India)
- Photo stability chamber (TH-90S, Thermo lab, Mumbai, India).

Development and Optimization of RP – HPLC Method:

a) Selection of Detection Wavelength:

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. Sultamicillin has λmax at 205 and 278nm with hunch 215nm in methanol. Wavelength of 215 nm was selected as it gives good absorption. Further, monograph of sultamicillin and sultamicillin tosilate and other reported methods also prescribe 215nm for HPLC test in related substances and assay.[22]

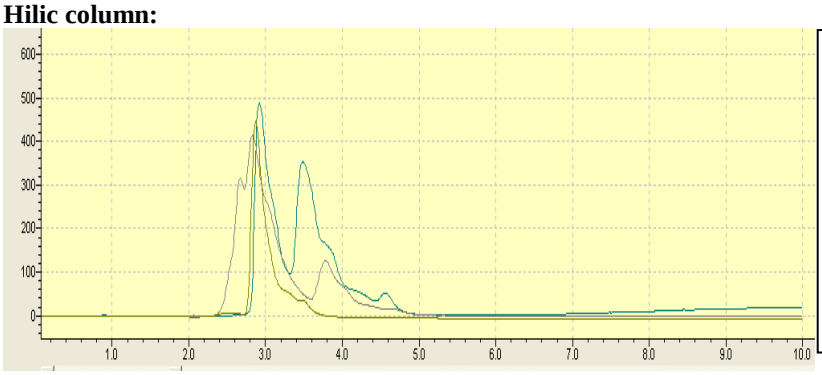
b) Selection of Column and Chromatographic Conditions:

Proper selection of the HPLC method depends upon the nature of the sample (ionic or ionisable or neutral molecule), its molecular weight, pKa and solubility. Selection of the proper column is the first step and selection of Mobile phase is the next step. To optimize the chromatographic conditions the effect of chromatographic variables such as mobile phase, pH, flow rate and solvent ratio were studied. Finally the chromatographic condition was chosen that gave the best resolution, symmetry and capacity factor for estimation of drug.[23]

c) Selection of Column:

To begin with 1mg/ml solution of sultamicillin, sulbactam and ampicillin were separately injected using different columns available at the institute to find out the optimum separation and resolution of sultamicillin. Different columns where tried like. C8, C18, Hilic, Phenyl hexyl. All the columns were of Phenomenex brand, the separation of sultamicillin from sulbactam and ampicillin was the goal. The retention time, and tailing factor and separation were observed.[24]

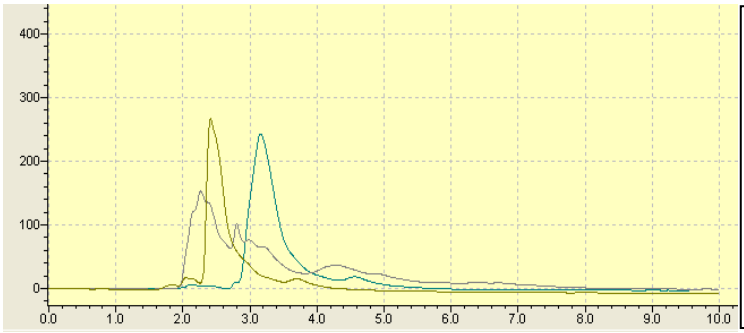
Hilic column:



Column selection
Column: Phenomenex Luna Hilic
Sultamicillin
Ampicillin
Sulbactum
All 3 peaks are overlapping

Figure-9: Sultamicillin is not separating from ampicillin and sulbactam.

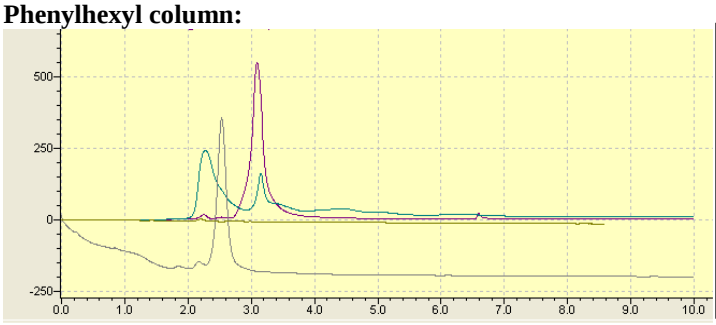
C8 Column:



Column selection
Column: Phenomenex Luna C8
Sultamicillin
Ampicillin
Sulbactum
Ampicillin and Sultamicillin are Overlapping

Figure-10: Sultamicillin is almost separating from sulbactam but not from ampicillin.

Phenylhexyl column:



Column: Phenomenex Luna PhenylHexyl
Sultamicillin
Ampicillin
Sulbactum
Separation was achieved between Sultamicillin and Sulbactum
But Ampicillin was not adequately separated from Sultamicillin

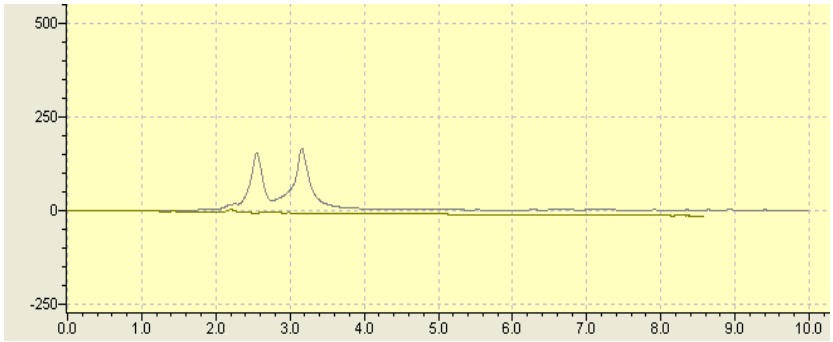


Figure-11: Sultamicillin is separated from sulbactam but not from ampicillin. when mixture of sultamicillin and sulbactam were injected, separation tookplace.

a) Selection of Mobil Phase:

Different organic solvent were used to bring about proper resolution of sultamicillin peak and separation from ampicillin and sulbactam, likely degradation products. ACN was found to be more suitable, than methanol bringing about resolution Phosphate buffer pH-3 employed in sultamicillin dehydrates tosilate related substances and assay test by BP was used as aqueous phase. The details of trials and chromatographic conditions tried are summarized in table.[25]

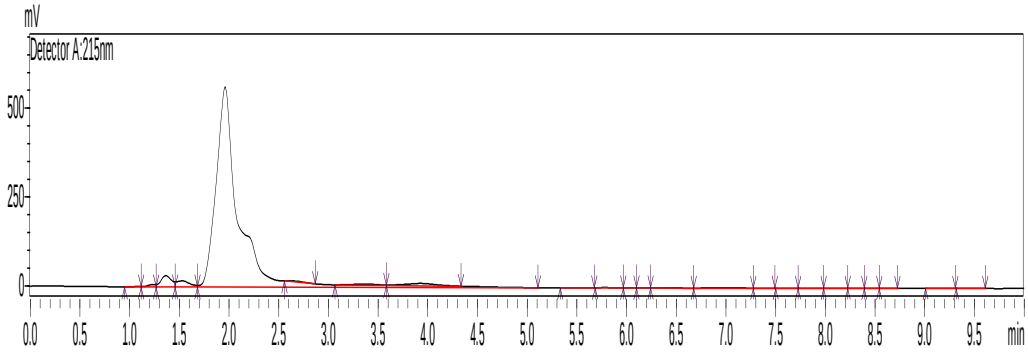
Optimization of Chromatographic Conditions

Table-6: Optimization of Chromatographic Conditions.

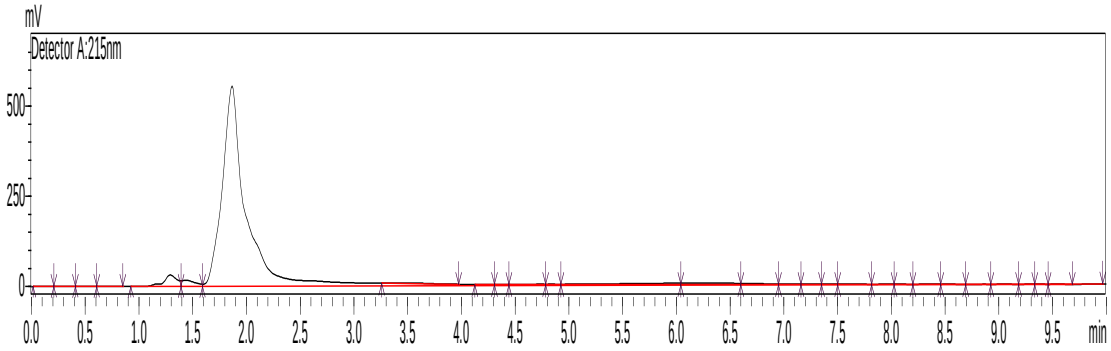
Trial	Chromatographic condition (Mobile Phase used)	Remarks
1	Buffer:Methanol (70:30 v/v)	Tailing and split peak
2	Buffer:Methanol (80:20 v/v)	Fronting and tailing both observed batter than above
3	Buffer:Methanol:ACN (70:20:10 v/v)	The peak at Rt 1.282min was well resoled but to the falling two peaks are margin not resolved
4	Buffer:ACN (70:30 v/v)	Satisfactory resolution with little tailing
5	Buffer:ACN (80:20 v/v)	Resolution retention and tailing improved, better than other experiments
6	Buffer:ACN (80:20:10 v/v)	Main peak broadened, Rt increased

Of the entire trials pH-3 phosphate buffer:ACN (80:20v/v) was found to be better.

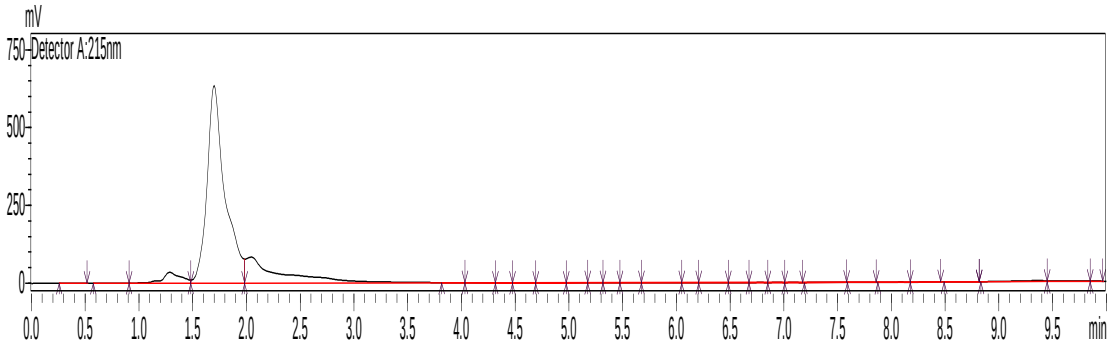
1. Buffer:Methanol (70:30):



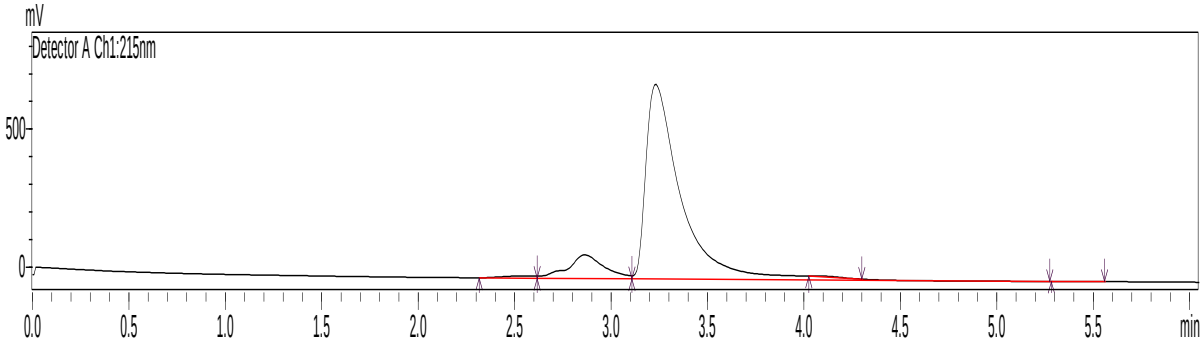
2. Buffer:Methanol (80:20):



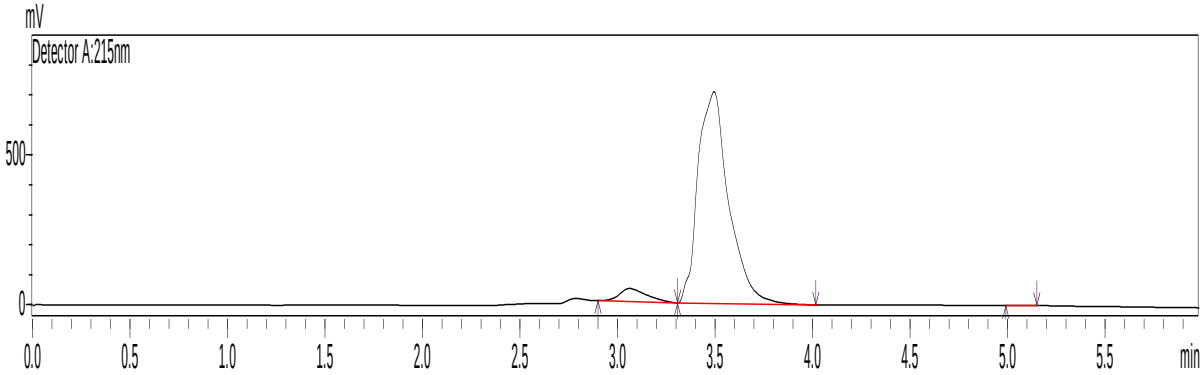
3. Buffer:Methanol/ACN (70:20:10):



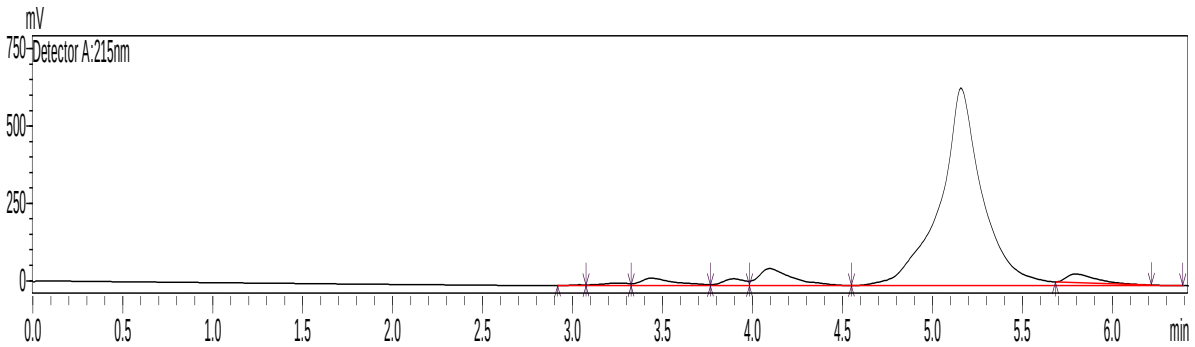
4. Buffer: ACN (70:30):



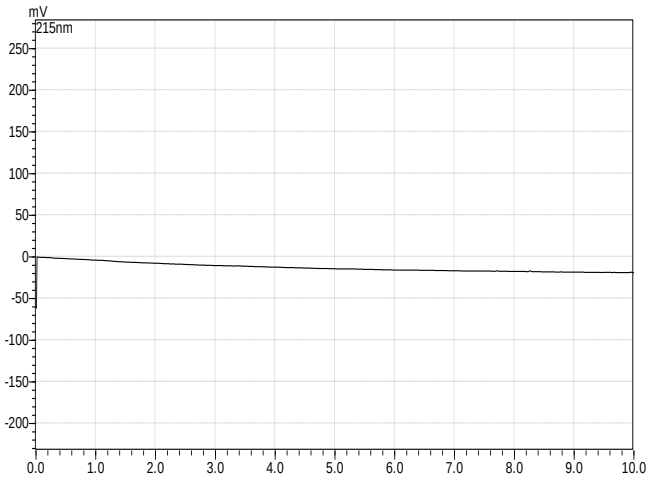
5. Buffer:ACN (80:20) 1ml:



6. Buffer: ACN (80:20) 0.8ml:



Sultamicillin Blank:



Sultamicillin standard:

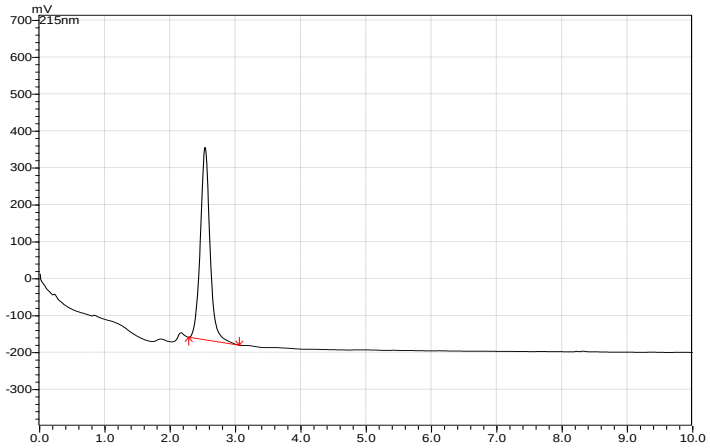


Figure-12: HPLC chromatogram

Sultamicillin test:

Chromatographic condition:

HPLC system: LC 2010 CHT (Shimadzu), PDA detector (PDA–SPD-M10AVP, Shimadzu)
Column: Hilic, Phenyl hexyl, 250×4.6mm, 5µ.
Mobile phase: 70:30 (Buffer:Acetonitrile)
Buffer: 1.050 gm KH_2PO_4 was dissolved in 700ml of water, pH was adjusted at 3.00 with orthophosphoric acid and volume was made up to 1liter with water and mixed (10mM)
Flow rate: 1.0 ml/min
Detection wavelength: 215nm
Injection volume: 10µL
Column Oven temp: 40ºC
Run time: 10 min
Diluents: Mixture of Water:ACN (80:20)
The peak immediately preceding peak of sultamicillin is mostly believed due to tosilate (toluene sulfonic acid)

System suitability data:

Sultamicillin peak was resolved at retention time of 2.53min with a resolution of 1.66 indicating required separation. Tailing factor of 1.112 was also good and number of theoretical plates was around 1500.[26]

Preparation of test (sample):

Marketed sample of sultamicillin 375mg tablets (sultam) was taken for analysis. Twenty tablets were weighed to determine average weight (0.602gm)the tablets were crushed and quantity of powder equivalent to 100mg (0.160gm)was transferred to 100ml volume flask and about 30ml methanol was added and sonicated for 10 minutes. The volume was made up to mark with methanol and mixed. Aliquot of 5ml was diluted to 10ml with methanol. This gives 500µg/ml concentration.[27]

Method Validation:

Linearity and range:

Overlain chromatogram of sultamicillin was Figure-13. The linearity of sultamicillin was found to be in the range of 200-1000µg/ml with correlation 0.999.

Table-7: Linearity data of sultamicillin.

Conc. (µg/ml)	Peak area mean (n=3)	SD	%RSD
200	432809	1040.83	0.24
400	917783	2730.07	0.29
600	1549931	8760.59	0.56
800	2161237	8185.35	0.38
1000	2907230	2124.02	0.073

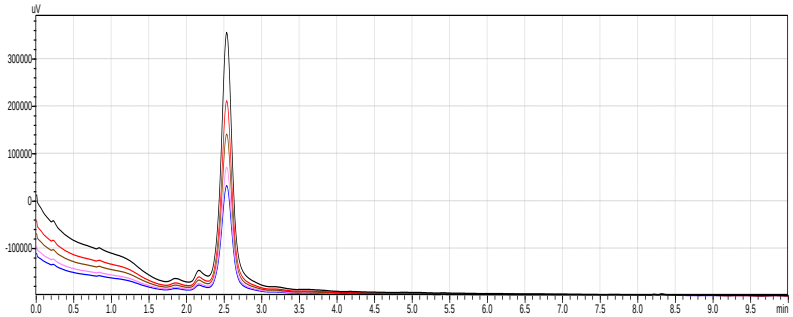


Figure-13: Overlain Linearity Chromatogram of Sultamicillin.

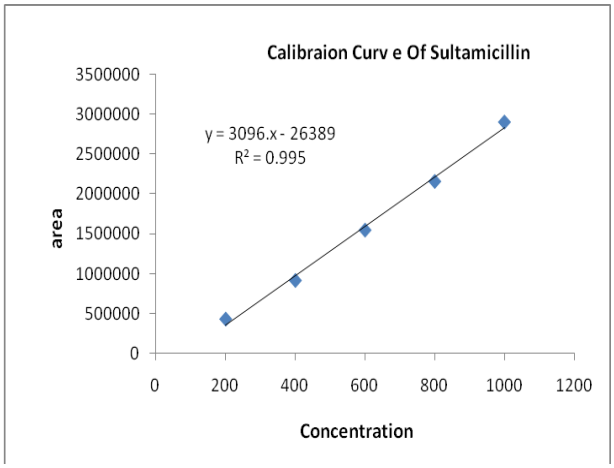


Figure-14: Calibration curve of sultamicillin.

Table-8: Linearity Results for sultamicillin.

Regression Analysis	Sultamicillin
Regration equation	Y= 3096x-26389
Correlation co-efficient	0.995
Slope	3096
Intercept	26389

Precision:

1. Repeatability (Intraday precision)

The data for Intraday precision for sultamicillin is shown in Table-9. The %RSD for Intraday precision was found to be 0.49-0.84% for sultamicillin.[28]

Interday precision (different days)

The data for Interday precision for sultamicillin is shown in Table-10. The %RSD Interday precision for was found to be 1.43-0.38% for sultamicillin.[29]

Table-9: Intraday precision data for sultamicillin.

Conc.(µg/ml)	Mean peak Area±SD (n=3)	%RSD
400	921376.3±5676.826	0.616125
500	1237172±6064.079	0.490156
600	1570431±13228.76	0.842365

Table-10: Interday precision data for sultamicillin.

Conc.(µg/ml)	Mean peak Area±SD (n=3)	%RSD
400	910734.3±8413.775	0.923845
500	1229502±17662.87	1.436587
600	935866.3±3579.572	0.382487

Accuracy:

Table-11: Accuracy data for Sultamicillin.

Test conc.	Std conc.	Mean peak area	Amount found	%Recovery±SD	%RSD
250	150	907806.3	395.65	99.62±1.127	1.13
250	250	1205825	488.64	97.29±0.55	0.568
250	350	1484038	574.49	96.25±0.601	0.628

LOD and LOQ:

The limit of detection (LOD) was found to be 2.01 ppm; while the limit of quantification (LOQ) was found to be 6.03 ppm for sultamicillin.[30]

Table-12: LOD and LOQ.

LOD	LOQ
2.01 ppm	06.03 ppm

Table-13: Summary of validation parameters of sultamicillin.

Sr. No.	Parameters	Sultamicillin
1	Linearity Range	Straight line
2	Regression equation	Y= 30961x-26389
3	Correlation co-efficient	0.995
4	Intraday	0.49-0.84%
	Interday	1.43-0.38 %
5	Limit of Detection	2.01 ppm
6	Limit of Quntitation	6.03 ppm

Tablet Assay:

Table-14: Tablet Assay of Sultamicillin.

Tablet Formulation	Label claim	%Assay±RSD
Sultamicillin	375mg	97.65±0.68

Robustness:

Table-15: Robustness of Sultamicillin.

		%Assay	SD	%RSD
As such		99.33	0.63	0.97
Flow Rate	0.8	97.68	0.76592	0.777
	1	98.51		
	1.2	99.21		
pH	2.8	95.32	0.976439	1.012
	3	100.01		
	3.2	96.86		
Organic solvent	81:19	97.9	1.579631	1.590499
	80:20	99.03		
	79:21	101.02		

Comparison of Development And Validated Analytical Method Of Sultamicillin:

t-Test for the development and validation method:

For the comparison of the two developed methods i.e. UV method and HPLC method for estimation of Sultamicillin tablet formulation by applying t-Test. Paired Two sample for means using Microsoft Excel - 2010 data analysis tool.[31,32]

Table-16: %Assay result of sultamicillin by developed methods.

Sr. No.	UV Method	HPLC Method
1	97.86	96.99
2	97.85	98.85
3	98.05	99.32
4	95.32	98.39
5	96.91	99.96
6	99.32	96.56
Mean	97.65	98.34

Table-17: Result of t-Test: paired Two Sample for Means for Sultamicillin.

Trials	Variable 1	Variable 2
Mean	97.65	98.34
Variance	1.34	2.91
Observation	6	6
Hypothesized Mean Difference	-1.59	-1.41
Df	10	10
T state	0.1-0.05	0.5-0.6
P(T<=t) one –tail	1.036	1.959
T critical one –tail	10%	90%
P(T<=t)two –tail	5%	80%
t critical two -tail	**UV=** 0.1<P>0.05	**HPLC**=0.5<P>0.6

The obtained values of t_{state} is lowered than $t_{critical}$ two tail, which leads to the conclusion that there is no significant difference are observed in assay results obtained by two methods. Statistical significance was performed by Student's t-test and found the t-value of UV (01.1-0.05) and P-value (0.1<P>0.05) and t-value for HPLC (0.5-0.6) and P-value (0.5<P>0.6). Hence any method can be used for estimation of Sultamicillin.

CONCLUSION

Sulbactam is hydrolyzed at acidic and alkaline pH to decompose ester link to form free ampicillin and free sulbactam. UV spectrophotomertic method was developed for estimation of sultamicillin in tablet dosage form. The drug exhibits well defined absorption maximum at 278 nm. RP HPLC test method was developed with a view to separate sultamicillin from ampicillin and sulbactam, the likely hydrolysis products. The developed method could satisfactorily separate sultamicillin from sulbactam but not from ampicillin. Of the four types of columns tried C8, C18, Hilic and Phenylhexyl, the Phenonenex make phenylhexyl column was found to be more suitable in achieving the separation. The method exhibited good linearity from 200-1000 µg/mL and was used at 500 ppm concentration for assay. The method has been validated for linearity, precision, accuracy, LOD, LOQ and robustness as per ICH guidelines. Both the methods compared well by paired students T test and can be used for estimation of the drug in bulk form and in tablet formulation. The method can be used for stability study of sultamicillin after making required adjustments in operating conditions to achieve base line separation from ampicillin.

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