RESEARCH ARTICLE

Analytical Method Development and Validation for Sultamicillin Tosylate Dihydrate in Bulk and Pharmaceutical Dosage Forms by RP-HPLC

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ABSTRACT

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the Sultamicillin Tosylate Dihydrate in bulk and pharmaceutical dosage form. In spectrophotometric method the stock and working standard solutions of the drugs were prepared in methanol. Sultamicillin Tosylate Dihydrate has shown maximum absorbance at 225 nm. The RP-HPLC method for Sultamicillin Tosylate Dihydrate was developed using phenomenex C18 column (150 mm × 4.6 mm, 5 μm) as stationary phase and Acetonitrile:Water (45:55) as mobile phase at 1.0ml/min flow rate and the method was validated in accordance with ICH guidelines. Sultamicillin Tosylate Dihydrate has linearity in the conc. range of 10-60µg/ml ($r^2$0.9991) in RP-HPLC method. Sultamicillin Tosylate Dihydrate was eluted at 6.9 min. Results of assay and validation studies were satisfactory. So, the developed analytical method can be successfully applied for the routine analysis of Sultamicillin Tosylate Dihydrate in pharmaceutical dosage forms.

KEYWORDS

Sultamicillin Tosylate Dihydrate, Method Validation, RP-HPLC

INTRODUCTION

Sultamicillin Tosylate Dihydrate, chemically known as (2S,5R)-(3,3-Dimethyl-4,4,7-trioxo-4-thia-1-azobicyclo[3.2.0]hept-2-ylcarbonyl)methyl(2S,5R,6R)-6-[(2R)-2-amino-2-phenylacetylamino]-3,3dimethyl-7-ox0-4-thia-1-azabicyclo[3.2.0]heptanes-2-carboxylatemono-4-tolunesulfonate dihydrate. This is a mutual (joint) prodrug of Ampicillin and Sulbactam compounds attached together with ester connection.

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This mutual prodrug is one of the antibiotics with plenty antimicrobial spectrum for the treatment of childhood pneumonia. The irretrievable β-lactamase inhibitor sulbactam has been combined chemically via ester linkages with ampicillin to form sultamicillin. It was composed of double esters of formaldehyde hydrate in which one of the hydroxyl groups is esterified with ampicillin and sulbactam. It is hydrolyzed quickly in neutral or faintly alkaline conditions, while hydrolyzed; it forms ampicillin and hydroxylmethyl sulbactam or sulbactam and hydroxylmethyl ampicillin by different routes.It is available obtainable in both oral and parenteral preparations for child (pediatric) use. Sultamicillin is also a valuable treatment option for a multiplicity of paediatric infections, bacterial infections in children

including those due to β-lactamase-producing organisms. The use of β-lactam and β-lactamase inhibitor mixtures, particularly ampicillin and sulbactam, as empiric treatment or prophylaxis for number of pediatric infections are healthy established, and have been extensively reviewed over number of years. The antimicrobial action of Sultamicillin had been established *in vitro* against extensive range of gram-positive and negative organisms and as well as anaerobes [2, 3].
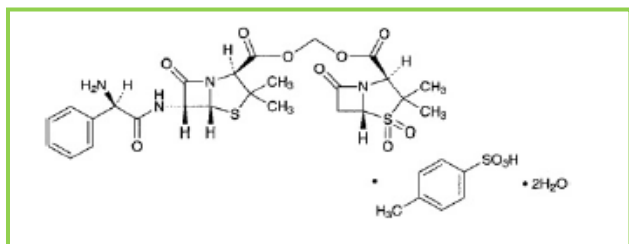

Fig.1: Chemical structure of Sultamicillin Tosylate Dihydrate[4]

Sultamicillin Tosylate Dihydrate is a white crystalline powder which is freely soluble in methanol, acetonitrile, acetone and insoluble in water, benzene, chloroform, diethyl ether.

The present work is to develop and validate RP-HPLC method for the determination of Sultamicillin Tosylate Dihydrate in API and its Pharmaceutical Dosage Form.

MATERIAL & METHODS

A calibrated weighing balance (Shimadzu) of 1 mg sensitivity was used.

A HPLC Younglin Acme 9000 series quaternary gradient pump SP 930D. HPLC system accomplished with UV 370D UV Visible detector with 20μl Rheodyne injector. Data was processed on Autochrome-3000 software. Column C18 (150 x 4.6 mm, 5μm) phenomenex with UV method analysis was performed on UV visible double beam spectrophotometer Shimadzu 1800.

Mobile phase filtered through a Nylon 6,6 membrane 0.45 μm, 47mm filters (pall India Pvt.Ltd. Mumbai) using vacuum pump. Ultra sonicator (Microlean-103) was used for degassing the mobile phase.

The solutions were filtered through 0.45 μ syringe filter (Phenomenex).

Chemicals

Sultamicillin Tosylate Dihydrate drug powder was gifted by Associated Biotech, Vill. Kishanpura, Gurumajra Road, Baddi, India. Sultamicillin Tosylate Dihydrate tablets of 375 mg strength were purchased from the local pharmacy in Solapur under commercially available brand name Marzon (Eris Lifesciences Limited).

Acetonitrile LiChrosolv®, water LiChrosolv® was purchased from Merck Specialities Pvt. Ltd, Mumbai.

Method

Chromatographic Conditions

The chromatographic separation was performed by analytical column: phenomenex C18 column (150 x 4.6 mm, 5μm) using mobile phase acetonitrile:water(45:55) at flow rate 1.0 ml/min. with isocratic elution. The injection volume was 20 μl and the run time was 10 minute. Detection was carried out at 225 nm.

Preparation of standard stock solution:

The standard stock solution of Sultamicillin Tosylate Dihydrate was prepared by transferring, accurately weighed 10 mg of Sultamicillin Tosylate Dihydrate to 10 ml of volumetric flask containing 5ml methanol and dissolved. Then volume was made up to the mark by using methanol to gives concentration 1000 μg/ml. From this 1ml of the solution was transferred to a 10 ml volumetric flask and make up the volume with mobile phase (ACN:Water) to get a concentration of 100 μg/ml of sultamicillin Tosylate Dihydrate and labelled as "Standard stock solution".

Tablet solution of Sultamicillin Tosylate Dihydrate

Tablet powder weight containing equivalent 10 mg of sultamicillin Tosylate was weighed and transferred to a 10 ml volumetric flask then dissolved in the methanol LR. The volume was made up to the mark with same solvent to

obtain conc. of 1000µg/ml of Sultamicillin Tosylate Dihydrate. From the resulting solution 1 ml was diluted to 10 ml with the ACN:Water (4.5:5.5) solvent to obtain conc. of 100µg/ml of Sultamicillin Tosylate Dihydrate , and labeled as 'Std Stock Tablet Sultamicillin Tosylate Dihydrate'.

Assay of sultamicillin Tosylate Dihydrate Tablet

20 tablets weighed and powdered. The powder equivalent to 10 mg of sultamicillin Tosylate dihydrate was weighed transferred into100 ml volumetric flask and dissolved in methanol LR. Solution was sonicated for 15 minutes and final volume was made up to the mark with methanol LR. 1ml of solution was transferred into 10 ml of volumetric flask and diluted up to 10ml with mobile phase and sample was analysed.

Selection of wavelength

The standard solution of 100µg/ml was scanned in the UV range 200-400nm. The solution showed maximum absorption at 225nm.

Validation of RP-HPLC Method

I. Specificity

The chromatogram of standard solution of Sultamicillin Tosylate Dihydrate was compared with chromatogram of its degradants.

II. Linearity

From the 'Std Stock Sultamicillin Tosylate Dihydrate' (100µg/ml) solution, the volume quantity of 1, 2, 4, 5 and 6 ml were transferred in a series of 10ml volumetric flasks. The volume was made up to the mark with mobile phase to obtain the concentration of 10, 20, 40, 50 and 60µg/ml of Sultamicillin Tosylate Dihydrate .

The solutions were filtered through syringe filter and 20µl injected into the HPLC system and their chromatogram were recorded for 10mins. Under the chromatographic conditions as described above after getting a stable baseline. Peak areas were recorded for all the peaks. Calibration curve of Sultamicillin Tosylate Dihydrate was constructed by plotting the peak area of Sultamicillin Tosylate Dihydrate *v/s* conc. of Sultamicillin Tosylate Dihydrate. The correlation coefficient (r^2) of least square linear regression for Sultamicillin Tosylate Dihydrate was calculated.

III. Range

The range of analytical method was decided from the interval between upper and lower level of calibration curves by plotting the curve. The correlation coefficient (r^2) of least square linear regression for Sultamicillin Tosylate Dihydrate was calculated.

IV. Precision

The precision of an analytical method was studied by performing Repeatability and intermediate precision.

a) Repeatability: From the 'Std Stock Sultamicillin Tosylate Dihydrate' (100µg/ml) solution, 2ml was transferred in 10ml volumetric flasks. The volume was made up to the mark with mobile phase to obtain the conc. of 20µg/ml of Sultamicillin Tosylate Dihydrate. The solution was filtered through syringe filter and 20µl injected into the HPLC system and its chromatogram was recorded under the same chromatographic conditions after getting a stable baseline. Peak area was recorded. The procedure was repeated for thrice.

V. Limit of Detection

Detection limit was determined based on the standard deviation of peak areas of same concentrations i.e. Standard solution of Sultamicillin Tosylate Dihydrate (20µglml) prepared six times and LOD calculated by the following formulae.

LOD = 3.3(SD/S)

Where, SD- Standard deviation; S- Slope of Curve

VI. Limit of Quantitation

Quantitation limit was determined based on the standard deviation of peak areas of same concentrations i.e. Standard solution of Sultamicillin Tosylate Dihydrate (20µglml)

prepared six times and LOQ calculated by the following formulae.

LOQ calculated by the following formulae.

LOQ = 10(SD/S)

Where, SD- Standard deviation; S- Slope of Curve

VII. Robustness

The standard solution of (20µg/ml) was prepared and analyzed at different flow rates (0.9, 1.0, 1.1 ml/min) and at different wavelengths (224, 225, and 226).

VIII. System Suitability

Sample solutions of Sultamicillin Tosylate Dihydrate (50µg/ml) were prepared and analyzed six times. Chromatograms were studied for different parameters such as tailing factor, resolution and theoretical plates to see that whether they comply with the recommended limit or not.

IX. Accuracy

Recovery study was carried out by standard addition method by adding the known amount of sultamicillin Tosylate dehydrate to preanalysed sample at three different conc. level i.e. 80%, 100%, 120% of assay conc. and percent recovery were calculated.

0.5 ml tablet solution was transferred to 4 different 10ml volumetric flasks (Labelled as blank, 80%, 100%, 120%) separately and 0, 1.6, 2, 2.4ml of 100µg/ml 'standard solution' was added respectively and the volume was made up to the mark with mobile phase. and these samples were analysed.

RESULTS AND DISCUSSION

Determination of wavelength of maximum absorption

The wavelength of maximum absorption was found to be 225 nm. Hence HPLC analysis was carried out at 225nm.
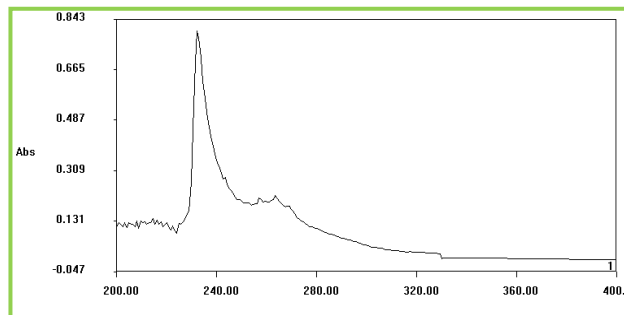

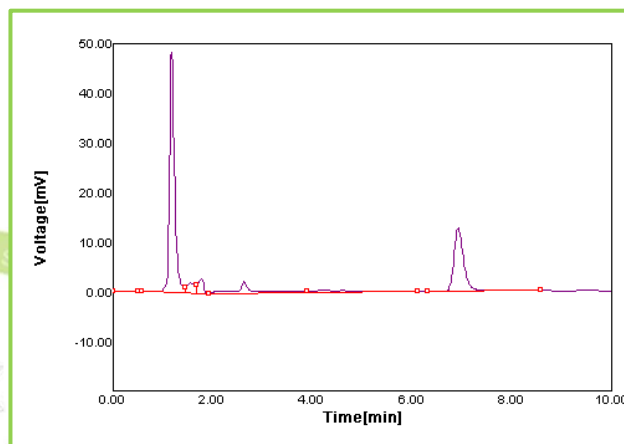Fig 2: Wavelength of maximum absorption of Sultamiciilin Tosylate

Specificity

Fig 3: Chromatogram of Sultamicillin Tosylate Dihydrate with degradants

Linearity

The linearity of this method was determined at the range from 10-60µg/ml for Sultamicillin Tosylate Dihydrate.

The regression equation was found to be Y=8.7304x+5.5409 be, r^2=0.9991.

Table No-1: Linearity table

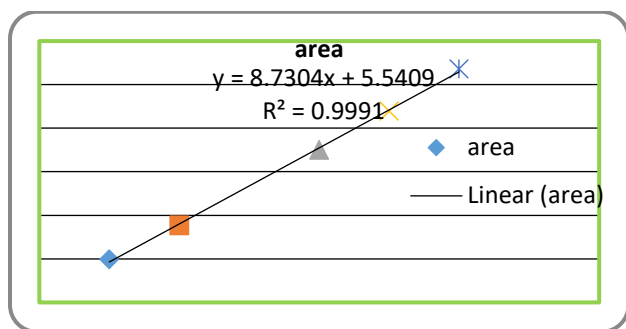| Sr. No	Concentration (µg/ml)	Peak Area(mV)
1	10	98.48
2	20	176.48
4	40	349.73
4	50	438.66
5	60	535.84

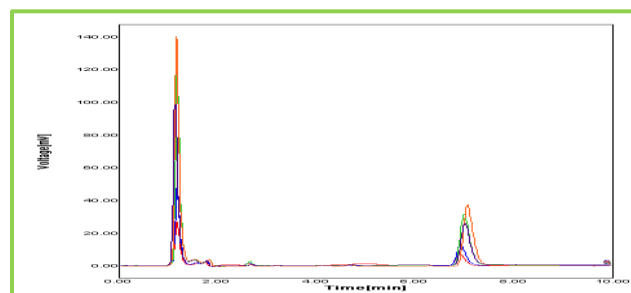Fig. 3: Linearity graph of Sultamicillin Tosylate Dihydrate

Fig.4: 0verlain chromatograms of serial dilutions of Sultamicillin Tosylate Dihydrate in optimized chromatographic conditions

The linearity for Sultamicillin Tosylate Dihydrate was found to be linear in the range of 10-60µg/ml with r^2= 0.9991 and the straight line equation as Y= 8.7304x+5.5409.

4.4 Range:

The range for RP-HPLC method for Sultamicillin Tosylate Dihydrate was found to be 10-60 µg/ml.

4.5 Precision

The precision was evaluated as the repeatability of the method and calculated as %RSD values for six determinations of peak area ratio performed on the same day and under the same experimental condition.

4.5.1 Repeatability

Table No-2: Repeatability study for Sultamicillin Tosylate Dihydrate

Injection	Peak Area of sultamicllin Tosylate Dihydrate (mV)
1	176.47
2	172.61
3	178.99
4	176.28
5	173.81
6	174.41
SD	2.284508
%RSD	1.30

The percentage RSD (<2) Values obtained shows that the method developed in précised at repeatability.

4.6 Limit of Detection

Detection limit is calculated based on standard deviation of response and slope

Table No-3: Limit of Detection Data of Sultamicillin Tosylate Dihydrate

Sultamicillin Tosylate Dihydrate	
LOD(µg/ml)	0.02754

4.7 Limit of Quantification

Quantification limit is calculated based on standard deviation of response and slope

Table No-4: *Limit* of Quantification data of Sultamicillin Tosylate Dihydrate

Sultamicillin Tosylate Dihydrate	
LOD(µg/ml)	0.08345

4.8 Robustness

The robustness was investigated by achieving deliberate changes in flow rate by ±1 units from 1.1 to 0.9ml/min and change in wavelength by ±1nm that flow is at 225nm Robustness of the method was carried out at concentration of 20µg/ml and then T, Rs and N were evaluated. The system suitability parameters remained unaffected over deliberate small change in the chromatoghraphic conditions, illustrating that the method was robust over an acceptable working range of its HPLC operational parameters.

Table No-5: Result of Robustness Study: Variation in flow rate and wavelength

Sr no.	Conditions	Range Investigated	Retention Time (min)	Theorotical Plates(N)	Resolution	Tailing Factor(T)
1	Flow Rate (ml/min)	1.1	6.15	7129.9	3.56	1.22
		0.9	7.58	8195.1	8.36	1.26
2	Wavelength (nm)	226	6.88	9493.3	7.91	1.25
		224	6.85	5013.8	2.138	1.08

System Suitability Testing

Study of resolution, tailing factor and capacity factor shows system is suitable for this method

Table No-5: Results of System Suitability Parameters

Analyte	Retention Time (min)	Tailing Factor (T)	Theoretical Plates (N)	Resolution (R)
Sultamicillin Tosylate Dihydrate	6.9	1.34	7349.6	7.64
Required limits	--	T < 2	N > 2000	R > 2

Assay of Sultamicillin Tosylate Dihydrate

Table No-6: Results of Assay

Tablet Formulation	Lable claim	Amount taken µg	Amount found µg	Assay%
Marzon	375 mg	37	38	102%

Accuracy

The accuracy study of method was determined through the recovery test of the samples, using known amounts of sultamicillin Tosylate dehydrate reference standard.

Table No-7: Results of accuracy of Sultamicillin Tosylate Dihydrate

Sr.no	Level of % Recovery	Amount of Tablet sample solution (ml)	Amount of standard drug added (µg/ml)	Amount Added µg	Amount Found (µg/ml)	% Recovery
1	0	0.5	0	0	0	-
2	80	0.5	1.6	16	16.28	101
3	100	0.5	2	20	20.74	103
4	120	0.5	2.4	24	24.75	103

SUMMARY AND CONCLUSION

Summary

Analytical method development was started with preliminary studies of the Sultamicillin Tosylate Dihydrate according to BP. The drug is freely soluble in methanol. The stock solutions of the drug were prepared in methanol.

The RP-HPLC method for estimation of Sultamicillin Tosylate Dihydrate dosage form was developed. The quantification was carried out by using Phenomenex C18 column (150 mm × 4.6 mm, 5 µm) as stationary phase and acetonitrile: water (45:55) as mobile phase. Mobile phase was maintained at a flow rate of 1.0ml/min. The UV detector was operated at 225 nm and Sultamicillin Tosylate Dihydrate eluted at 6.90 min.

The developed RP-HPLC method can be successfully applied for the routine analysis of Sultamicillin Tosylate Dihydrate.

Table No-8: Summary of RP-HPLC Method of Sultamicillin Tosylate Dihydrate

Sr. No.	Parameters	Sultamicillin Tosylate Dihydrate
1.	Linearity Range (µg/ml)	10-60
2.	Regression Equation (y = mx+c)	8.730x+5.540
3.	Correlation Coefficient (r^2)	0.999
4.	LOD (µg/ml)	0.02754
5.	LOQ (µg/ml)	0.08345
6.	Repeatability (%RSD)	1.06
7.	Robustness(%RSD) Flow Rate Wavelength	11.10% 23.94%

Conclusion

In conclusion, the proposed HPLC method is simple, accurate, reproducible method for estimation of Sultamicillin Tosylate Dihydrate in bulk and pharmaceutical formulation. This method shows Assay of Sultamicillin Tosylate Dihydrate within the specified limit. The method shows no interference by the excipients. The statistical parameters and recovery data reveals the good accuracy and precision of the proposed method. Finally, since no pharmacopoeial method for determination of Sultamicillin Tosylate Dihydrate in bulk and pharmaceutical formulations have been reported yet, the proposed method could be useful and suitable for the estimation of the Sultamicillin Tosylate Dihydrate in bulk and pharmaceutical dosage forms.

ACKNOWLEDGEMENT:

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