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RESEARCH ARTICLE

UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF SULTAMICILLIN TOSYLATE IN API AND IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Simple, rapid, sensitive, precise and specific UV Spectrophotometric for the determination of Sultamicillin Tosylate in API and pharmaceutical dosage form were developed and validated. In this method solutions of Sultamicillin Tosylate were prepared in Methanol. Sultamicillin Tosylate standard solution was scanned in the UV range (400-200nm) in a 1cm quartz cell in a double beam UV spectrophotometer. The standard solution of Sultamicillin Tosylate showed maximum absorption at wavelength 232 nm. The method obeys Beer's law in the concentration range from 20-100µg/ml. The correlation coefficient was found to be 0.999 and regression of the curve was found Y=0.004x+0.052 with excellent recovery 97-102%. Limit of detection and limit of quantification were found to be 3.689µg/ml and 11.18µg/ml respectively. The ruggedness and robustness was performed. The method was validated for several parameters like accuracy, precision as per ICH guidelines. Statistical analysis proved that the methods are repeatable and specific for determination of the said drug. These methods can be adopted in routine assay analysis of Sultamicillin Tosylate in API and pharmaceutical dosage form.

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INTRODUCTION

Sultamicillin Tosylate, 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-phenyl acetylamino]-3, 3dimethyl-7-ox0-4-thia-1-azabicyclo [3.2.0] heptanes-2-carboxylatemono-4-tolunesulfonate dihydrate. Which is a mutual (joint) prodrug of Ampicillin and Sulbactam compounds attached to gether with ester connection. 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 hydroxyl methyl sulbactam or sulbactam and hydroxyl methyl 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 pediatric infections, bacterial infections in children including those due to β-lactamase-producing organisms.

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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 (Sadhana et al., 2013; Vundavilli et al., 2011). Fig.1: Chemical structure of Sultamicillin Tosylate [Japanese Pharmacoepoeia 15 Edition]. Sultamicillin Tosylate it 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 UV Spectrophometric Method for The Determination of Sultamicillin Tosylate in API and its Pharmaceutical Dosage Form with help of Methanol solvent.

MATERIALS AND METHODS

Instruments

For weighing, a calibrated weighing balance (Shimadzu) of 1 mg sensitivity was used. A Systronic UV-visible double beam spectrophotometer- 2201 was used. All other glasswares and apparatus were made up of borosilicate and were calibrated.

Chemicals

API- Sultamicillin Tosylate is pure drug was gifted by Associated Biotech, Vill. Kishanpura, Gurumajra Road, Baddi, India.

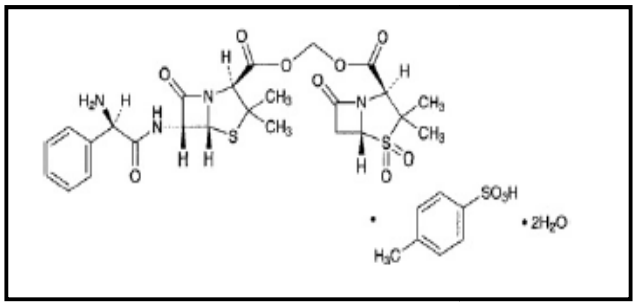

Fig.1. Chemical structure of Sultamicillin Tosylate [Japanese Pharmacoepoeia 15 Edition]

Tablets of 375 mg strength were purchased from the local pharmacy in Solapur under commercially available brand name Marzon (Eris Lifesciences Limited), Methanol LR was used in this study.

UV Spectroscopic Method:

Solvent selection

Sultamicillin Tosylate is soluble in Methanol. In the present investigation Methanol was selected as a solvent.

Preparation of standard stock solution

The standard stock solution of Sultamicillin Tosylate was prepared by transferring, accurately weighed 10 mg of Sultamicillin Tosylate to 10 ml of volumetric flask containing 5ml Methanol. Dissolve drug properly. Then volume was made up to the mark by using Methanol to gives concentration 1000 µg/ml. From this 5ml of the solution was transferred to a 50 ml volumetric flask and make up the volume with Methanol to give a concentration of 100 µg/ml which is a standard stock solution and it is further diluted with Methanol to get concentration range of 20-100 µg/ml.

Determination of Absorption Maxima

The standard stock solution of 100µg/ml was scanned in the range of 400-200 nm to determine the wavelength of Maximum Absorption. The drug showed Absorption maxima at 232 nm.

Preparation of Calibration Curve

For the preparation of calibration curve, the concentration of 20-100µg/ml were prepared by pipetting out 2, 4, 6, 8 and 10 ml of the 100 µg/ml solution into 10 ml volumetric flask and made up the volume with Methanol.

The absorbance of each solution was measured at 232 nm against Methanol as a blank. Calibration curve of the Sultamicillin To sylate was plotted by taking the absorbance obtained on the y-axis and the concentration of the solution on the x-axis. The curve showed linearity in the range of 20-100 µg/ml with correlation coefficient 0.999.

Quantitative analysis of pharmaceutical tablet dosage form

Twenty tablets were weighed accurately and powdered. Powder equivalent to 10 mg sultamicillin Tosylate was weighed and transferred to a 10 ml volumetric flask. It was dissolved in 10 ml Methanol and sonicate for 15 minutes to get

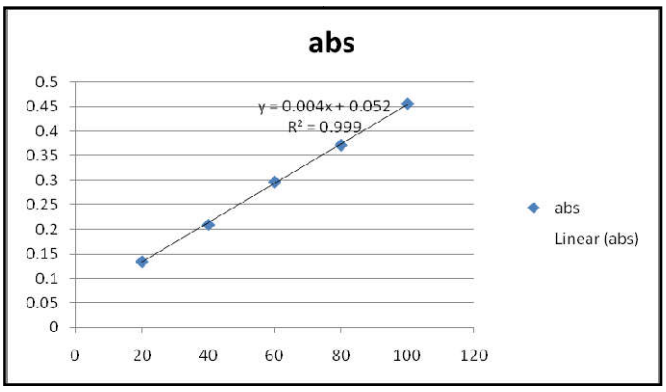

Fig. 2. Calibration curve of Sultamicillin Tosylate

a homogeneous solution. Then it was filtered through a 0.45 µ What man filter paper. A final concentration of 1000 µg/ml of Sultamicillin Tosylate was prepared. This solution was filtered through filter paper to remove some un- dissolved excipients. After filtration, from this 1ml was taken and diluted to 10 ml with Methanol which gives 100 µg/ml solution and the absorbance of the solution was measured at 232 nm.

Table No-1: Results obtained in the determination of Sultamicillin Tosylate in tablet dosage form

Tablet Formulation	Label claim	Amount Taken	Amount found	Assay %
Marzon	375 mg	100µg/ml	99 µg/ml	99%

Method validation

The developed method was validated as per ICH guidelines for following Parameters

- Linearity: 2, 4, 6, 8, 10 ml of Standard solution were transferred in a series of 10 ml volumetric flasks. The volume was made up to the mark with Methanol to obtain the concentration of 20, 40, 60, 80, 100 µg/ml. Then absorption of these solutions were recorded and the graph was plotted of absorption against concentration. The correlation coefficient (r^2) of least square linear regression of Sultamicillin Tosylate was calculated.
- Range: The Range of the analytical method was decided from the interval between upper and lower level of calibration curve by plotting curve.
- Accuracy: Recovery study was carried out by standard addition method by adding known amount of Sultamicillin Tosylate to the preanalyzed sample at three different concentration levels that is 80%, 100%, 120% of assay concentration and percent recovery were calculated.
- 2 ml of tablet solution was transferred to 4 different 10 ml volumetric flasks (labelled as blank, 80%, 100%, 120%) separately and 0, 1.6, 2, 2.4 ml of 100 µg/ml standard solution was added respectively and the volume was made up to the mark with Methanol. Absorbances were noted for these samples.
- Standard deviation and %RSD was calculated. Accuracy is reported as % recovery which was calculated from expression as equation given below,
- % Recovery = $\frac{\text{Observed value}}{\text{True value}} \times 100$

- Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scattering) between a series of measurements obtained from multiple sampling of the same sample under the prescribed conditions. The precision of the method was determined in terms of repeatability and intraday and interday precisions.
- Intraday and interday precision (Intermediate Precision):
- Intraday precision was determined by analyzing the drugs at concentrations (60µg/ml) and each concentration for three times, on the same day.
- Interday precision was determined similarly, but the analysis being carried out daily, for two consecutive days.
- Repeatability
- Repeatability of the method was determined by analyzing six samples of same concentrations of the drug (60µg/ml). Absorbance of each was measured.
- Robustness
- The robustness of the developed method is its capacity to remain unaffected by small changes in altered conditions. To determine the robustness of the method, the wavelength of analysis was deliberately and the assay was evaluated. The effect of detection wavelength was studied at ±5 nm.
- Ruggedness
- Ruggedness was determined by carrying out analysis by two different analysts and the respective absorbance was noted and the results were indicated as % RSD.
- Limit of Detection: Detection limit was determined based on standard deviation of absorbance of same concentration that is standard solution of Sultamicillin Tosylate (60µg/ml) prepared six times and LOD calculated by
- LOD = 3.3(SD/S)
- Where, SD- standard deviation; S= slope of curve
- Limit of Quantification: Quantification limit was determined based on the standard deviation of peak area of same concentration that is standard solution Sultamicillin Tosylate (60µg/ml) prepared six times and LOQ calculated by

LOD = 10(SD/S)
Where, SD= standard deviation; S= slope of curve

RESULTS

Determination of wavelength of maximum absorption:

The wavelength of maximum absorption was found to be 232 nm.

Linearity

The linearity of this method was determined at ranging from 20-100 µg/ml for Sultamicillin Tosylate.

The regression equation was found to be Y=0.004x+0.052 be, r^2=0.999. The linearity for Sultamicillin Tosylate was found to be linear in the range of 20-100µg/ml with r^2= 0.999 and the straight line equation as Y= 0.004x+0.052.

Accuracy

The accuracy of the analytical method for Sultamicillin Tosylate was determined at 80%, 100% and 120% levels of standard solution.

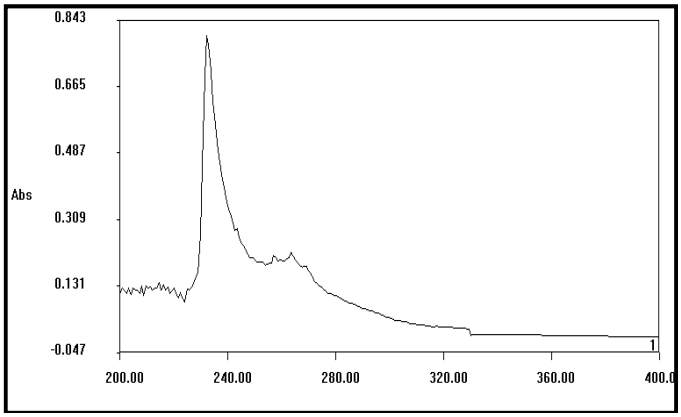

Fig. 3. Wavelength of maximum absorption of Sultamiciilin Tosylate

Table No 2. Linearity table

Sr. No	Concentration (µg/ml)	Absorbance
1	20	0.135
2	40	0.210
4	60	0.297
4	80	0.372
5	100	0.456

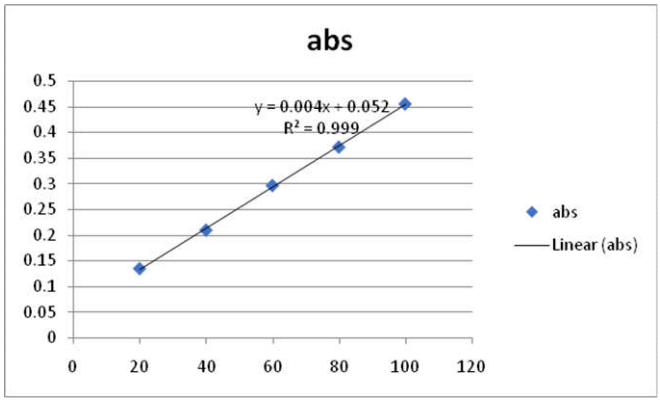

Fig. 4. Linearity graph of Sultamicillin Tosylate

Absorbance was measured at 232 nm and results were expressed in terms of % recoveries.

Precision

The precision (measurement of intraday, interday, repeatability) results showed good reproducibility with the present relative standard deviation (% RSD) was below 2.0 %. This indicated that method was highly precise.

Ruggedness

Ruggedness was determined by carrying out analysis by two different analysts and the respective absorbance was noted and the results were indicated as % RSD.

Table No 3. Table for accuracy

Sr. No	Level of % Recovery	Amount of Tablet sample (ml)	Amount of standard drug added (µg/ml)	Amount added µg	Amount found (µg/ml)	% Recovery
1	0	2	0	0	0	
2	80	2	1.6	36	35.25	97.91%
3	100	2	2	40	39.75	99.37%
4	120	2	2.4	44	45.25	102s%

Table No 4. Intraday morning precision

Sr. no	Concentration (µg/ml)	Absorbance	SD	% RSD
1	60	0.277		
2	60	0.272		
3	60	0.282	0.004472	1.61%
4	60	0.277		
5	60	0.272		
6	60	0.282		
		$\bar{y}$=0.277		

Table No 5. Intraday afternoon precision

Sr. no	Concentration (µg/ml)	Absorbance	SD	% RSD
1	60	0.329		
2	60	0.320		
3	60	0.326	0.004099	1.26%
4	60	0.329		
5	60	0.32		
6	60	0.326		
		$\bar{y}$ =0.325		

Table No 6. Intraday evening precision

Sr. no	Concentration (µg/ml)	Absorbance	SD	% RSD
1	60	0.326		
2	60	0.332		
3	60	0.336	0.004502	1.36%
4	60	0.332		
5	60	0.336		
6	60	0.326		
		$\bar{y}$ =0.331		

Interday Precision

Table no-7. Interday morning precision study

Sr. no	Concentration (µg/ml)	Absorbance	SD	% RSD
1	60	0.346		
2	60	0.342		
3	60	0.348		
4	60	0.342	0.002714	0.79%
5	60	0.341		
6	60	0.344		
		$\bar{y}$ =0.343		

Table no 8. Interday afternoon precision study

Sr. no	Concentration (µg/ml)	Absorbance	SD	% RSD
1	60	0.392		
2	60	0.390		
3	60	0.388	0.002168	0.55%
4	60	0.394		
5	60	0.393		
6	60	0.392		
		$\bar{y}$ =0.391		

Table no-9. Interday evening precision study

Sr. no	Concentration (µg/ml)	Absorbance	SD	% RSD
1	60	0.383		
2	60	0.385		
3	60	0.380		
4	60	0.381	0.003225	0.83%
5	60	0.387		
6	60	0.388		
		$\bar{y}$ =0.384		

Repeatability

Table No-10. Repeatability study

Sr. no	Concentration (µg/ml)	Absorbance	SD	% RSD
1	60	0.277		
2	60	0.272		
3	60	0.282	0.004472	1.61%
4	60	0.277		
5	60	0.272		
6	60	0.282		
		$\bar{y}$ =0.277		

Limit of Detection

Table No-11. For Limit of Detection

LOD (µg/ml)	3.689 µg/ml

Limit of Quantification

Table No 12. For Limit of Quantification

LOQ (µg/ml)	11.18 µg/ml

Robustness

Table No-13. Robustness study

Sr. No	Wavelength (nm)	Absorbance	SD	% RSD
1	227	0.267		
2	228	0.285		
3	229	0.305		
4	230	0.315		
5	231	0.321	0.018487	6.02%
6	232	0.329		
7	233	0.326		
8	234	0.318		
9	235	0.309		
10	236	0.301		
11	237	0.299		
		$\bar{y}$ =0.30681		

Ruggedness

Ruggedness was determined by carrying out analysis by two different analysts and the respective absorbance was noted and the results were indicated as % RSD.

Table no 14. For Ruggedness

Analyst-1		
Concentration(µg/ml)	Absorbance	Statistical analysis
60	0.300	
60	0.300	Mean=0.299833
60	0.300	SD=0.000408
60	0.300	%RSD=0.136%
60	0.300	
60	0.299	
Analyst-2		
60	0.278	
60	0.278	Mean=0.278167
60	0.279	SD=0.000753
60	0.279	%RSD=0.270%
60	0.277	
60	0.278	

Table No 15. For Summary

Sr. No	Parameters	Values
1	Beer's law limit(µg/ml)	20-100
2	Absorption maxima (nm)	232
3	Standard Regression Equation	Y=0.004x +0.052
4	Correlation Coefficient (r^2)	0.999
5	Accuracy	97-102%
6	Precision (%RSD)	
	Repeatability	1.61%
7	LOD	3.689 µg/ml
8	LOQ	11.18µg/ml
9	Robustness (%RSD)	6.02%
10	Ruggedness(%RSD)	0.136075 and 0.270700
11	Assay (%)	99%

DISCUSSION

Preliminary Analysis of Sultamicillin Tosylate

Preliminary analysis of Sultamicillin Tosylate such as description, solubility was performed.

UV-spctrophotometry for Sultamicillin Tosylate

Sultamicillin Tosylate being UV absorbing has been successfully employed for its quantitative determination by UV Spectrophotometric method. Being soluble in Methanol, stock solutions and working standards were made in Methanol. The maximum wavelength of absorption of drug was determined by taking scan of the drug solution in the UV region (200-400 nm). The correlation of the standard curve for the drug was 0.999. The accuracy was from 97-102% at 232 nm. The proposed method showed absorption maxima at 232nm and obeyed Beer's law in the concentration of 20-100 µg/ml. The limit of detection (LOD) was found to be 3.689 µg/ml and limit of quantification (LOQ) to be 11.18µg/ml respectively. All statistical data prove validity of proposed method, which can be applied for routine analysis of Sultamicillin Tosylate.

Assay of tablet formulation

Amount of drug present in tablet formulation was calculated using equation at 267.2nm, and Y=0.004x +0.052 and amount of Sultamicillin Tosylate was found to be 99% of label claim respectively. This method can be employed for routine analysis of Sultamicillin Tosylate.

Summary and conclusion

Summary of UV Spectrophotometeric Method of Sultamicillin Tosylate.

Conclusion

The UV-spectrophotometric method was developed and it is found to be simple, accurate, precise, highly sensitive, reproducible and inexpensive. The proposed method was found suitable for determination of Sultamicillin Tosylate in API and its pharmaceutical dosage form without any interference from the excipients. This method can be effectively applied for the routine analysis of Sultamicillin Tosylate in API. Its advantages are the low cost of reagents, speed and simplicity of sample treatment, satisfactory precision and accuracy.

Abbreviations

UV: Ultra Violet
µg: Microgram
Nm: nanometer
ml: mililiter
API: Active Pharmaceutical Ingredient
%: Percentage

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