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DEVELOPMENT AND VALIDATION OF UV-SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF SULBACTAM SODIUM IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

The aim of the present work is to develop sensitive, simple, accurate, precise and cost-effective UV-spectrophotometric method for the determination of Sulbactam sodium, an β-lactamase inhibitor, it is used in combination with β-lactam antibiotics. A simple UV-spectrophotometric method was developed using Phosphate buffer (pH 7.4) as the solvent with a wavelength of 213 nm. The calibration graph was linear over the range of 5-25 g/ml in the methods a correlation coefficient (r) of 0.99978. The apparent molar absorptivity value was 2.2970×105. The other parameters such as limit of detection (LOD), limit of quantification (LOQ) values are also reported. The accuracy and precision of the methods were evaluated based on intra-day and inter-day variations. The accuracy of the methods was further confirmed by standard addition procedure. The proposed methods were successfully applied to the determination of Sulbactam sodium in dry injection and the results obtained are comparable with the official method.

KEYWORDS: Sulbactam sodium, UV-spectrophotometry, Phosphate buffer (pH 7.4).

INTRODUCTION

Ultraviolet and visible (UV-Vis) absorption spectroscopy is the measurement of the attenuation of a beam of light after it passes through a sample or after reflection from a sample surface. The visible spectrum ranges from 400 nm to about 800 nm. The colour we see depends on wavelength. The colour of a substance is determined by which colours of light it absorbs and which colour it transmits or reflects (the complementary colours. Colour is an important property of a substance. The colour of matter is related to its absorptivity or reflectivity. The human eye sees the complementary colour to that which is absorbed.

Spectroscopy is related to the interaction of light with matter. As light is absorbed by matter, the result is an increase in the energy content of the atoms or molecules. When ultraviolet radiations are absorbed, this results in the excitation of the electrons from the ground state towards a higher energy state. Molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb energy in the form of ultraviolet light to excite these electrons to higher anti-bonding molecular orbitals. The more easily excited the electrons, the longer the wavelength of light it can absorb. There are four possible types of transitions (π–π*, n–π*, σ–σ*, and n–σ*), and they can be ordered as follows: σ–σ > n–σ > π–π* > n–π*. The absorption of ultraviolet light by a chemical compound will produce a distinct spectrum which aids in the identification of the compound.

Single component analysis

The assay of an absorbing substance can be carried out quickly by preparing its solution in a transparent solvent and measuring its absorbance at a suitable wavelength. The wavelength normally selected is the wavelength of maximum absorbance (λmax).The concentration of the absorbing substance is then calculated from the measured absorbance using one of the following three principal procedures.

1. Use of Standard Absorptivity Value

The use of the standard absorptivity value ($A^{1\%}_{1}cm$) or molar absorptivity (ε) avoids the need to prepare a standard solution of the reference substance. This method is advantageous in situations where it is difficult or expensive to obtain a sample of the reference substance.

The following equations can be used:

$$A = A^{1\%}_{1cm}$$

or A = εbc

Where:

- A = Absorbance
- $A^{1\%}_{1cm}$ = Specific absorbance of a 1% solution measured in a 1 cm cell
- ε = Molar absorptivity
- b = Path length of the cell (cm)
- c = Concentration of the solution

2. Use of Calibration Graph

The absorbance of several standard solutions of the reference substance at different concentrations are prepared. A graph is plotted with concentration on the X-axis and absorbance on the Y-axis. The sample to be analysed is prepared at a concentration within the standard range, using the same procedure as that used to prepare the standard solutions. The absorbance of the sample is measured, and its concentration is determined by extrapolation using the standard graph or calibration graph. Calibration data are essential for confirming compliance with Beer–Lambert's law. If the graph is non-linear, i.e., the absorbance is not proportional to the concentration, it may indicate an error in the method. Possible causes include slight variations in pH, temperature, and heating time.

3. Single- or Double-Point Standardisation

(a)Single-Point Standardisation

This involves measuring the absorbance of a sample solution and a standard solution of the reference substance. The standard and sample solutions are prepared in a similar manner. The concentration of the standard solution should be close to that of the sample solution.

The concentration of the sample is calculated using the proportional relationship.

$$C_{test} = \frac{A_{test} \times C_{std}}{A_{std}}$$

Where

- Ctest = Concentration of the test solution
- Cstd = Concentration of the standard solution
- Atest = Absorbance of the test solution
- Astd = Absorbance of the standard solution

$$\frac{C_{std}}{A_{std}} = \frac{C_{test}}{A_{test}}$$

$$C_{test} = \frac{A_{test} \times C_{std}}{A_{std}}$$

This procedure is used for the assay of substances that obey Beer–Lambert's law and for which a reference standard of adequate purity is available.

(b) Double-Point Standardisation

In this method, two standard solutions are prepared, one with a higher concentration than the sample and the other with a lower concentration than the sample. The concentration of the substance in the sample solution is calculated using the following equation.

$$C_{test} = \frac{(A_{test} - A_{std1})(C_{std1} - C_{std2}) + C_{std1}(A_{std1} - A_{std2})}{A_{std1} - A_{std2}}$$

Where

- Ctest = Concentration of the test solution
- Atest = Absorbance of the test solution
- Cstd1= Concentration of the higher standard solution
- Cstd2 = Concentration of the lower standard solution
- Astd1 = Absorbance of the higher standard solution
- Astd2 = Absorbance of the lower standard solution

VALIDATION METHODS

Method validation of impurities in drugs ensures that analytical methods (mostly HPLC/UHPLC) accurately detect and quantify trace-level impurities or degradation products according to ICH Q2(R1) guidelines. Key parameters include specificity, accuracy, precision, linearity, range, detection limit (LOD), quantification limit (LOQ), and robustness. These steps ensure compliance for regulatory, stability, and quality control studies.

Core Validation Parameters for Impurities as per ICH Q2(R1) guidelines, the following parameters must be evaluated for quantitative and limit tests of impurities.

Specificity/Selectivity: Demonstrates that the method can measure the impurities in the presence of the active pharmaceutical ingredient (API), excipients, and degradation products.

Linearity and Range: Establishes that the detector response is proportional to the concentration of impurities, typically from the LOQ to 120% or 150% of the specification limit.

Accuracy (Recovery): Assesses how close the test results are to the true value, usually performed at three concentrations (e.g., LOQ, 100%, and 150% of the specification limit).

Precision: Evaluates the closeness of agreement between a series of measurements. Includes **Repeatability (System Precision):** Multiple measurements of the same sample under the same conditions.

Intermediate Precision (Ruggedness): Assesses within-laboratory variations (different days, analysts, or instruments).
Detection Limit (LOD): The lowest amount of analyte that can be detected, but not necessarily quantified.
Quantification Limit (LOQ): The lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy.
Robustness: Measures the capacity of the method to remain unaffected by small, deliberate variations in parameters (e.g., pH, flow rate, temperature, change in wavelength).

Sulbactam Sodium
Sulbactam is a semisynthetic β-lactamase inhibitor belonging to the penicillanic acid sulfone class. It is used in combination with β-lactam antibiotics to inhibit bacterial β-lactamase enzymes and restore the activity of antibiotics against resistant organisms. Sulbactam acts as a β-lactamase inhibitor. Binds irreversibly to bacterial β-lactamase enzymes.

Forms a stable acyl-enzyme complex (suicide inhibition), permanently inactivating the enzyme. Prevents hydrolysis of β-lactam antibiotics, thereby restoring their antibacterial activity. Has weak intrinsic antibacterial activity against Acinetobacter spp. Protects β-lactam antibiotics (e.g., ampicillin, cefoperazone) from enzymatic degradation and enhances their antibacterial effect.

Sulbactam Sodium is soluble in water, dil acid, methanol, ethanol, dimethylsulphoxide and phosphatebubffer of pH 7.4.

MATERIALS AND METHODS

1. MATERIALS

1. Apparatus

The Spectrophotometric measurements were carried out using Shimadzu Pharmaspec 1700 UV/Visible spectrophotometer.

2. Materials and reagents

All chemicals used were of analytical reagent grade. A sample of Sulbactam Sodium was purchased from Glemmark, Mumbai.

2. METHOD DEVELOPMENT

1. Preparation of Phosphate buffer pH 7.4

Pour 800 ml water into a beaker add 20.214 g dibasic sodium phosphate and 3.394 g monobasic sodium phosphate stir to dissolve. Adjust the pH with HCl or NaOH 1 M to 7.4. Pour the solution into volumetric flask and make up to 1000 ml with distilled water.

2. Determination of λ max

A standard solution of Sulbactam Sodium was prepared by dissolving 50 mg of the sample in 50 ml of Phosphate buffer. The prepared solution was scanned in the ultraviolet (UV) region ranging from 210 nm to 221 nm using a UV-Visible spectrophotometer to determine the wavelength of maximum absorbance (λmax). The spectrum obtained showed a distinct absorption maximum (λmax) at 213 nm.

3. Preparation of standard calibration curve

A stock solution of Sulbactam Sodium was prepared by dissolving 50 mg of the sample in 50 mL of Phosphate buffer to obtain a concentration of 1000 µg/ml. From this stock solution, a series of standard dilutions were prepared in the concentration range of 5-25 µg/ml. Phosphate buffer was used as the blank. Each of the prepared dilutions was scanned at the previously determined λmax of 213 nm using a UV-Visible spectrophotometer, and the corresponding absorbance values were recorded. A calibration curve was then plotted between concentration (µg/ml) and absorbance.

4. Estimation of Sulbactam sodium in injection

For the estimation of Sulbactam Sodium from its formulation, 50 mg of the powdered sample was accurately weighed and transferred into a clean standard flask. The powder was dissolved in 50 ml of Phosphate buffer with continuous stirring to ensure complete dissolution. The solution was then filtered using Whatman filter paper. From the filtrate, 5 ml was pipetted out from the stock solution and diluted to 100 ml. To get a concentrations of 5 to 25 µg/ml pipetted out 1,2,3,4,5 ml of sample solution and diluted to 10 ml by using phosphate buffer. The absorbance of the resulting solution was measured at the selected wavelength 213 nm with Phosphate buffer as a blank. The concentration of the drug in each solution was determined by single point standardisation.

$$C_{test} = \frac{A_{test}\ X\ C_{std}}{A_{std}}$$

Amount of Sulbactam sodium in the given injection was calculated by the formula

$$\text{Amount of drug present} = \frac{\textit{Concentration of the test x average weight}}{\textit{Weight taken x dilution factor}}$$

Percentage labelled claim was calculated by the formula

$$\%\ \text{Labelled Claim} = \frac{\textit{Amount of drug present x 100}}{\textit{Labelled claim}}$$

3. ANALYTICAL METHOD VALIDATION

Validation of an analytical procedure ensures that the method used is accurate, precise, and reliable for its intended purpose. It confirms that the analytical results are consistent and reproducible. Method validation is essential in analytical development to optimize resources and ensure the method meets all required performance parameters.

1. Robustness

The robustness of the developed UV spectrophotometric method was evaluated by making deliberate changes in the analytical parameter wavelength to assess the stability of the method under minor variations. The absorbance of the standard solution 0f Sulbactam

Sodium (1000 µg/ml) was measured at three different wavelengths 210 nm, 213 nm, 221 nm.

2. Ruggedness

The degree of reproducibility of test results when a method is used under a variety of normal, real-world conditions, such as different analysts, instruments, laboratories, or days. Take 5 test tubes. Take 15 µg/ml of standard solution of Sulbactam Sodium in all the 5 test tubes. Measure the absorbance at different time by different analyst. Calculate the standard deviation.

3. Precision Study

The precision of the method was evaluated by measuring the absorbance of each concentration five times under identical conditions. The results showed minimal variation, confirming the reproducibility of the method.

4. Limit of Detection (LOD)

To determine the LOD, a diluted solution was prepared from the 1000 µg/ml stock solution of Sulbactam Sodium. The lowest concentration giving a measurable absorbance was detected at 213 nm.

5. Limit of Quantitation (LOQ)

To determine the LOQ of the method, a series of diluted solutions were prepared from the 1000 µg/mL stock solution of Sulbactam Sodium. Increasingly lower concentrations were prepared and their absorbance was measured at 213 nm. The concentration that produced a consistent and measurable absorbance with acceptable precision was considered as the LOQ.

6. Recovery studies

The accuracy, precision, specificity and validity of the present method was satisfied by conducting percentage recovery studies. A known quantity of the drug was added to the pre-analysed sample formulation and the contents were analysed by the proposed method.

The percentage recovery was calculated by using the following formula.

% Recovery = (Practical Value)/(Theoretical Value)×100

7. Linearity

A linear correlation was found between absorbance at 213 nm and concentration of Sulbactam sodium. From the regression equation: Y = a + bX (where Y = absorbance of drug solution; a = intercept; b = slope and X = concentration of drug in µg ml−1). The slope (b), intercept (a), correlation coefficient (r) and standard deviation of slope were calculated.

Correlation coefficient = (n Σ xy-(Σx)(Σy))/([nΣx^2-(Σx)^2][nΣy^2-(Σy)^2])

Where,

n = Total number of standard data points

Σx = Sum of all concentration value

Σy = Sum of all response value

Σxy = Sum of the product of each x and y pair

Σx2 = Sum of the squared concentration value

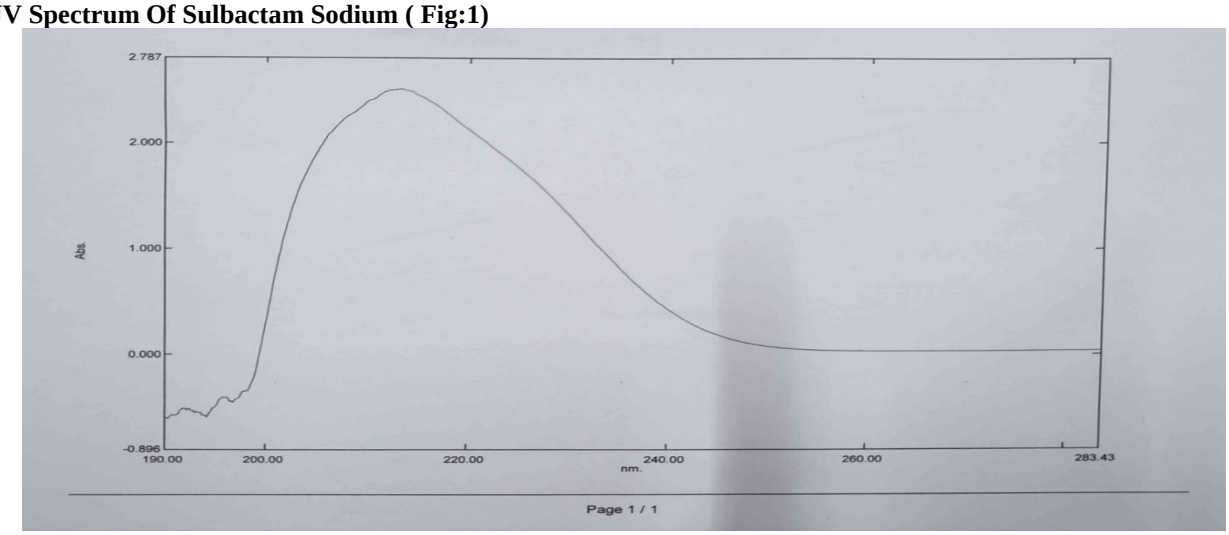
Σy2 = Sum of the squared response value

RESULT AND DISCUSSION

Determination of λmax

A standard solution of Sulbactam Sodium was prepared by dissolving 50 mg of the sample in 50 mL of Phosphate buffer. The prepared solution was scanned in the ultraviolet (UV) region ranging from 210 nm to 221 nm using a UV-Visible spectrophotometer to determine the wavelength of maximum absorbance (λmax). The spectrum obtained showed a distinct absorption maximum (λmax) at 213 nm. Fig.: 1

UV Spectrum Of Sulbactam Sodium (Fig:1)



Preparation of standard calibration curve

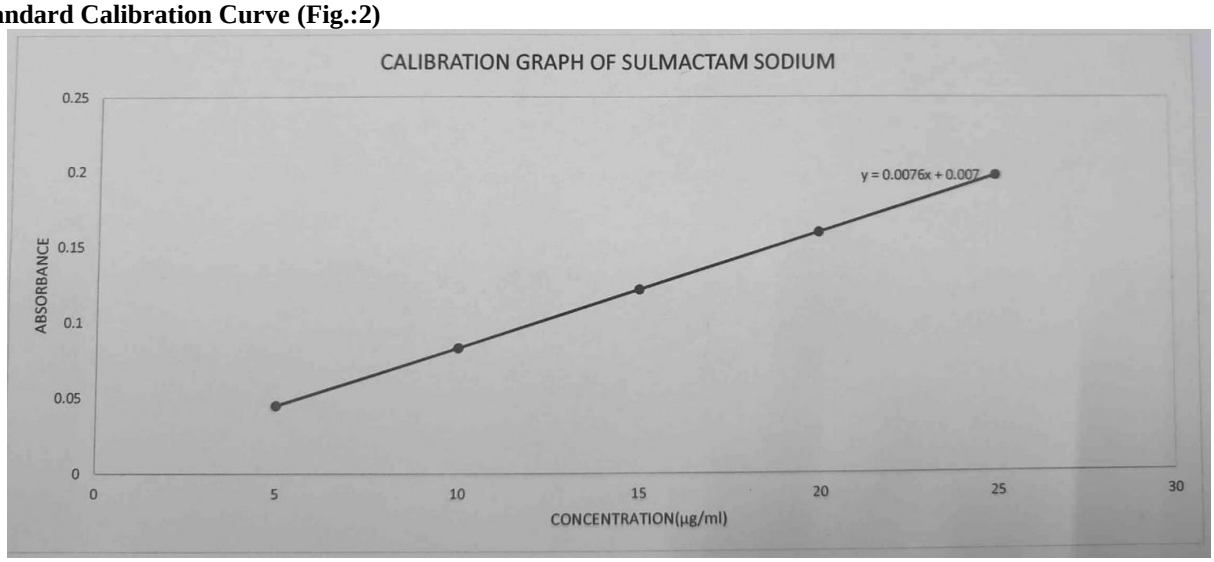
A stock solution of Sulbactam Sodium was prepared by dissolving 50 mg of the sample in 50 mL of Phosphate buffer to obtain a concentration of 1000 µg/ml. From this stock solution, a series of standard dilutions were prepared in the concentration range of 5-20 µg/ml. Phosphate buffer was used as the blank. Each of the prepared dilutions was scanned at the previously determined λmax of 213 nm using a UV-Visible spectrophotometer, and the corresponding absorbance

values were recorded. A calibration curve was then plotted between concentration (µg/ml) and absorbance. Table: 1;Fig.:2

Absorbance Values For Sulbactam Sodium (TABLE: 1)

CONCENTRATION(µg/ml)	ABSORBANCE
5	0.045
10	0.083
15	0.121
20	0.159
25	0.197

Standard Calibration Curve (Fig.:2)



Estimation of Sulbactam sodium in injection

For the estimation of Sulbactam Sodium from its formulation, 50 mg of the powdered sample was accurately weighed and transferred into a clean standard flask. The powder was dissolved in 50 ml of Phosphate buffer with continuous stirring to ensure complete dissolution. The solution was filtered, then pipetted out 5 ml of the solution and makeup to 100 ml by using buffer. From this solution 1,2,3,4,5 ml was pipetted out from the sample solution and diluted to 10 mL to get a concentration of 5 to 25 µg/mL with the same solvent. The absorbance of the resulting solution was measured at the selected wavelength 213 nm with Phosphate buffer as blank. The concentration of the drug in each solution was determined by single point standardisation. Table: 2

$$C_{test} = \frac{A_{test} \times C_{std}}{A_{std}}$$

Amount of Sulbactam sodium in the given injection was calculated by the formula

Amount of drug present = $\frac{\text{Concentration of the test x average weight}}{\text{Weight taken x dilution factor}}$

Percentage labelled claim was calculated by the formula

% Labelled Claim = $\frac{\text{Amount of drug present x 100}}{\text{Labelled claim}}$

Estimation Of Sulbactam Sodium In Injection(TABLE: 2)

SL.NO.	Astd	Atest	Cstd (µg/ml)	Ctest (µg/ml)	Labelled claim(mg)	Amount Present(mg)	% labelled claim
1	0.045	0.046	5	5.100	1000	1020	102
2	0.083	0.084	10	10.120	1000	1012	101.2
3	0.121	0.122	15	15.124	1000	1008	100.8
4	0.159	0.160	20	20.120	1000	1007	100.7
5	0.197	0.198	25	25.100	1000	1004	100.4

S.D = ±0.6180

Analytical Method Validation

Robustness

The absorbance of the standard solution (1000 µg/mL) was measured at three different wavelengths 210 nm, 213 nm, 221 nm. The results show that the absorbance value was maximum at 213 nm and decreased considerably at 210 nm and 221 nm. This indicates that small variations around the λmax do not significantly affect the results within a narrow range, confirming the robustness of the analytical method. Table:3.

Robustness (TABLE:3)

Wavelength (nm)	Absorbance
210	0.199
213	0.202
221	0.164

Ruggedness

The degree of reproducibility of test results when a method is used under a variety of normal, real-world conditions, such as different analysts, instruments, laboratories, or days. Take 5 test tubes. Take 15 µg/ml of standard solution in all the 5 test tubes. Measure the absorbance at different time by different analyst. Calculate the standard deviation. (Table:4)

Precision Study

The precision of the method was evaluated by measuring the absorbance of each concentration five times under identical conditions. The results showed minimal variation, confirming the precision of the method. Table:4

Precision Study (TABLE:4)

Concentration	1	2	3	4	5	S.D
5µg/mL	0.055	0.044	0.048	0.048	0.056	±0.004827
10µg/mL	0.091	0.083	0.086	0.080	0.084	±0.012920
15µg/mL	0.128	0.121	0.123	0.113	0.112	±0.006800
20µg/mL	0.165	0.159	0.162	0.147	0.140	±0.010630
25µg/mL	0.204	0.197	0.201	0.182	0.168	±0.530100

Limit of Detection

To determine the LOD, a diluted solution was prepared from the 1000 µg/ml stock solution. The lowest concentration giving a measurable absorbance was detected at 213 nm. The concentration was found to be 2µg/ml.

Limit of Quantitation

To determine the LOQ of the method, a series of diluted solutions were prepared from the 1000 µg/ml stock solution. Increasingly lower concentrations were prepared and their absorbance was measured at 213 nm. The concentration that produced a consistent and measurable absorbance with acceptable precision was considered to be 5 µg/ml.

Recovery Studies

The accuracy, precision, specificity and validity of the present method was satisfied by conducting percentage recovery studies. A known quantity of the drug was added to the pre-analysed sample formulation and the contents were analysed by the proposed method. Table:5

The percentage recovery was calculated by using the following formula.

$$\% \text{ Recovery} = \frac{\text{Practical Value}}{\text{Theoretical Value}} \times 100$$

Recovery Studies (TABLE:5)

Concentration(µg/ml)	Practical value	Theoretical value	% Recovery
10	0.085	0.083	102.4
20	0.160	0.159	100.6

Linearity

A linear correlation was found between absorbance at 213 nm and concentration of Sulbactam sodium. From the regression equation: $Y = a + bX$ (where Y = absorbance of drug solution; a = intercept; b = slope and X = concentration of drug in µg ml^{-1}). The slope (b), intercept (a), correlation coefficient (r) and standard deviation of slope were calculated. (Table:6 &7)

$$\text{Correlation coefficient} = \frac{n\,\Sigma\,xy-(\Sigma x)(\Sigma y)}{[n\Sigma x^2-(\Sigma x)^2][n\Sigma y^2-(\Sigma y)^2]}$$

Were,

n = Total number of standard data points

Σx = Sum of all concentration value

Σy = Sum of all response value

Σxy = Sum of the product of each x and y pair

Σx^2 = Sum of the squared concentration value

Σy^2 = Sum of the squared response value

Regression Equation (TABLE:6)

Parameter	Obtained values
Intercept	0.01498
Slope	0.00698
Correlation coefficient	0.99978
Standard deviation of intercept	±0.008224
Standard deviation of slope	±0.000855
Beer's law limits	5-20 µg/ml
Molar Absorptivity	2.2970×10^5

Correlation Coefficient (TABLE:7)

SL NO	Σx	Σy	Σxy	Σx^2	Σy^2	Correlation coefficient(r)
1	75	0.643	11.505	1375	0.096531	0.990
2	75	0.605	10.975	1375	0.087645	1.003
3	75	0.620	11.207	1375	0.091474	0.990
4	75	0.575	10.350	1375	0.192972	0.968
5	75	0.448	9.800	1375	0.070560	0.997

CONCLUSION

A sensitive, simple, accurate, precise and cost-effective UV Spectrophotometric method was developed for the estimation of Sulbactam sodium in injection. In the estimation of Sulbactam sodium, the drug sample was diluted in Phosphate buffer. A λ max of 213 nm was obtained.

Various concentrations ranging from 5-25 µg/ml showed linearity. This was considered as Beer's – Lamberts range.

Aliquot amount of Sulbactam Sodium was taken from the injection samples. This was diluted to get concentration from 5-20 µg/ml as that of the standard concentration. The absorbance was measured at 213 nm. The concentration was determined by single point standardisation method.

The method was Robustness, Rugged, Precise and showed good linearity. The Correlation coefficient was found to be 0. 99978. The Molar Absorptivity was found to be 2.29707×10^5.

This method gave a satisfactory label claim and recovery value. So, it can be used for routine analysis of the drug.

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