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#### DEVELOPMENT AND VALIDATION OF SIMULTANEOUS ESTIMATION OF SULBACTUM

#### AND AMPICILLIN (SULTAMICILLIN)

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Article history:	Abstract:					
Received: 27 <sup>th</sup> July 2015	Two new, simple, sensitive and economical UV spectrophotometric methods were developed for the simultaneous analysis of Sulbactum and Ampicillin in bulk and in pharmaceutical formulations (SULTAMICILLIN).					
Received in revised form:						
14 <sup>th</sup> August 2015	Analytic method development and validation are continuous and interconnected activities conducted throughout the drug development process. The practice of					
Accepted: 21 <sup>st</sup> August 2015	validation verifies that a given method measures a parameter as intended and establishes the performance limits of the measurement. Although apparently					
Available online:	contradictory, validated methods produce results within known uncertainties.					
25 <sup>th</sup> September 2015	These results are crucial to continuing drug development, as they define the emerging knowledge base supporting the product.					
*Corresponding author:	The time and effort that are put into developing scientifically-sound, robust, and transferrable analytic methods should be aligned with the drug development stage. The re-sources that are expended on method validation must be constantly					
Madhvi Ambekar	balanced with regulatory requirements and the probability for product					
Email:	commercialization. Method I is based on solving simultaneous equation. Sulbactum and Ampicillin show					
madhviambekar77@gmail.com	absorbance maximums at 272 and 332 nm respectively, so absorbance was					
Present address:	measured at the same wave lengths for the estimation of Sulbactum and Ampicillin. Method II is based on determination of Q-value. Both drugs (Sulbactum and Ampicillin) obey the Beer Lambert's law in the concentration range of 5-30 $\mu$ g/mL and the correlation coefficient values were found to be 0.9994 and 0.999 respectively Methods are validated according to ICH guidelines and can be adopted for the routine analysis of Sulbactum and Ampicillin in pure and tablet dosage form of SULTAMICILLIN. The developed methods were validated and from the statistical					
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These authors have no conflict of interest to declare.	data, it was found that the methods were linear, accurate and precise and can be successfully applied for the analysis of pharmaceutical formulations without interference of excipients. The proposed method is simple, selective and sensitive.					
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All rights reserved	Key words: Sulbactum, Ampicillin, estimation, ICH, validation					

# **1. INTRODUCTION**

Analytic method development, validation, and transfer are key elements of any pharmaceutical development program. This technical brief will focus on development and validation activities as applied to drug products. Often considered routine, too little attention is paid to them with regards for their potential to contribute to overall developmental time and cost efficiency.

These method-related activities are interrelated. They are iterative, particularly during early drug development phases. Parts of each process may occur concurrently or be refined at various phases of drug development. Changes encountered during drug development may require modifications to existing analytic methods. These modifications to the methods, n turn, may require additional validation or transfer activities, as shown below.

Effective method development ensures that laboratory resources are optimized, while methods meet the objectives required at each stage of drug development. Method validation, required by regulatory agencies at certain stages of the drug approval process, is defined as the "process of demonstrating that analytical procedures are suitable for their intended use". Method transfer is the formal process of assessing the suitability of methods in another laboratory. Each of these processes contributes to continual improvement of the methods and results in more efficient drug development.

Analytic methods are intended to establish the identity, purity, physical characteristics and potency of the drugs that we use. Methods are developed to support drug testing against specifications during manufacturing and quality release operations, as well as during long-term stability studies. Methods may also support safety and characterization studies or evaluations of drug performance. According to the International Conference on Harmonization (ICH), the most common types of analytic procedures are: It begins in the early phases of drug development as a set of informal experiments that establish the soundness of the method for its intended purpose. It is expanded in intensity and extent throughout the regulatory submission process into a fully-documented report that is required by NDA submission at Phase III and in support of commercial production. It is repeated whenever there is a significant change in instrumentation, method, specifications, and process, if applicable.

#### 1.1. Identification

Suitable identification tests should be able to discriminate between compounds of closely related structures which are likely to be present. The discrimination of a procedure may be confirmed by obtaining positive results (perhaps by comparison with a known reference material) from samples containing the analyte, coupled with negative results from samples which do not contain the analyte. In addition, the identification test may be applied to materials structurally similar to or closely related to the analyte to confirm that a positive response is not obtained. The choice of such potentially interfering materials should be based on sound scientific judgement with a consideration of the interferences that could occur.

#### **1.2.** Assay and Impurity Test(s)

For chromatographic procedures, representative chromatograms should be used to demonstrate specificity and individual components should be appropriately labelled. Similar considerations should be given to other separation techniques.

Critical separations in chromatography should be investigated at an appropriate level. For critical separations, specificity can be demonstrated by the resolution of the two components which elute closest to each other.

In cases where a non-specific assay is used, other supporting analytical procedures should be used to demonstrate overall specificity. For example, where a titration is adopted to assay the drug substance for release, the combination of the assay and a suitable test for impurities can be used.

The approach is similar for both assay and impurity tests:

### **1.2.1 Impurities are available**

For the assay, this should involve demonstration of the discrimination of the analyte in the presence of impurities and/or excipients; practically, this can be done by spiking pure substances (drug substance or drug product) with appropriate levels of impurities and/or excipients and demonstrating that the assay result is unaffected by the presence of these materials (by comparison with the assay result obtained on unspiked samples).

For the impurity test, the discrimination may be established by spiking drug substance or drug product with appropriate levels of impurities and demonstrating the separation of these impurities individually and/or from other components in the sample matrix.

#### **1.2.2 Impurities are not available**

If impurity or degradation product standards are unavailable, specificity may be demonstrated by comparing the test results of samples containing impurities or degradation products to a second well-characterized procedure e.g.: pharmacopoeial method or other validated analytical procedure (independent procedure). As appropriate, this should include samples stored under relevant stress conditions: light, heat, humidity, acid/base hydrolysis and oxidation.

# 1.3 Linearity

A linear relationship should be evaluated across the range (see section 3) of the analytical procedure. It may be demonstrated directly on the drug substance (by dilution of a standard stock solution) and/or separate weighing of synthetic mixtures of the drug product components, using the proposed procedure. The latter aspect can be studied during investigation of the range. Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. If there is a linear relationship, test results should be evaluated by appropriate statistical methods, for example, by calculation of a regression line by the method of least squares. In some cases, to obtain linearity between assays and sample concentrations, the test data may need to be subjected to a mathematical transformation prior to the regression analysis. Data from the regression line itself may be helpful to provide mathematical estimates of the degree of linearity.

# 1.4 Range

The specified range is normally derived from linearity studies and depends on the intended application of the procedure. It is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing amounts of analyte within or at the extremes of the specified range of the analytical procedure.

The following minimum specified ranges should be considered:

- for the assay of a drug substance or a finished (drug) product: normally from 80 to 120 percent of the test concentration;
- for content uniformity, covering a minimum of 70 to 130 percent of the test concentration, unless a wider more appropriate range, based on the nature of the dosage form (e.g., metered dose inhalers), is justified;

# 1.5 Accuracy

Accuracy should be established across the specified range of the analytical procedure.

# 1.5.1 Assay

# • Drug Substance

Several methods of determining accuracy are available:

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- a) application of an analytical procedure to an analyte of known purity (e.g. reference material);
- b) comparison of the results of the proposed analytical procedure with those of a second well-characterized procedure, the accuracy of which is stated and/or defined (independent procedure, see 1.2.);
- c) accuracy may be inferred once precision, linearity and specificity have been established.

# **1.5.2 Impurities (Quantitation)**

Accuracy should be assessed on samples (drug substance/drug product) spiked with known amounts of impurities.

In cases where it is impossible to obtain samples of certain impurities and/or degradation products, it is considered acceptable to compare results obtained by an independent procedure (see 1.2.). The response factor of the drug substance can be used.

It should be clear how the individual or total impurities are to be determined e.g., weight/weight or area percent, in all cases with respect to the major analyte.

# 1.5.3 Recommended Data

Accuracy should be assessed using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range (e.g., 3 concentrations/3 replicates each of the total analytical procedure).

Accuracy should be reported as percent recovery by the assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence intervals.

# 1.6 Precision

Validation of tests for assay and for quantitative determination of impurities

includes an investigation of precision.

# 1.6.1 Repeatability

Repeatability should be assessed using:

 a) a minimum of 9 determinations covering the specified range for the procedure (e.g., 3 concentrations/3 replicates each);

or

b) a minimum of 6 determinations at 100% of the test concentration.

# **1.6.2 Intermediate Precision**

The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. The applicant should establish the effects of random events on the precision of the analytical procedure. Typical variations to be studied include days, analysts, equipment, etc. It is not considered necessary to study these effects individually. The use of an experimental design (matrix) is encouraged.

# 1.6.3 Reproducibility

Reproducibility is assessed by means of an interlaboratory trial. Reproducibility should be considered in case of the standardization of an analytical procedure, for instance, for inclusion of procedures in pharmacopoeias. These data are not part of the marketing authorization dossier.

# 1.6.4 Recommended Data

The standard deviation, relative standard deviation (coefficient of variation) and confidence interval should be reported for each type of precision investigated.

# **1.7 Detection Limit**

Several approaches for determining the detection limit are possible, depending on whether the procedure is a non-instrumental or instrumental. Approaches other than those listed below may be acceptable.

# **1.8 Quantitation Limit**

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Several approaches for determining the quantitation limit are possible, depending on whether the procedure is a non-instrumental or instrumental. Approaches other than those listed below may be acceptable.

#### **1.9 Robustness**

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.

If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. One consequence of the evaluation of robustness should be that a series of system suitability parameters (e.g., resolution test) is established to ensure that the validity of the analytical procedure is maintained whenever used.

Examples of typical variations are :

- stability of analytical solutions;
- ➢ extraction time.

In the case of liquid chromatography, examples

of typical variations are :

- influence of variations of pH in a mobile phase;
- influence of variations in mobile phase composition;
- different columns (different lots and/or suppliers);
- temperature;
- flow rate.

In the case of gas-chromatography, examples of typical variations are: - different columns (different lots and/or suppliers);

- ➢ temperature;
- $\succ$  flow rate.

### 1.10 System Suitability Testing

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated. See Pharmacopoeias for additional information

S.				
No.	Parameter	Definition		
		an assessment of the difference between the measured value		
1.	Accuracy	and the real value		
		a measure of the agreement for multiple measurements on the		
2.	Precision	same sample		
		the ability to assess the analyte when in the presence of other		
3.	Specificity	components		
	Limits of			
	detection and	the lowest amounts of analyte that can be detected / determined		
4.	quantitation	accurately, respectively		
	Linearity and	the proportionality of the measurement to the concentration of		
5.	Range	the analyte within a specified range		
		a check of the effect of deliberate small changes to the method		
6.	Robustness	on the results		

Table 1.1: Parameters those are applicable to most methods

# EXPERIMENTAL PROCEDURES AND METHODOLOGY

# Solvent selection

Drug sulbactum is freely soluble in water  $(\geq 18 \text{ mg/mL})$ , freely soluble in 1 M HCl (50 mg/ml), sparingly soluble in methanol and very slightly soluble in ethanol (0.99mg/ml) and practically in soluble in acetonitrile & chloroform.

Drug ampicillin is soluble in water (10 mg/ml), freely soluble in 1 M HCl (50 mg/ml), soluble in methanol, sparingly soluble in ethanol, and practically insoluble in chloroform.

#### Preparation of phosphate buffer (pH 6.8)

Accurately weigh about 0.896 gm of NaOH, 6.804 gm of K<sub>2</sub>HPO<sub>4</sub>, dissolve in distilled water and make up the volume to 1Litre with distilled water.

# Preparation of standard stock solution (1000µg/mL)

Accurately weighed quantity of pure Sulbactum (10mg) and pure Ampicillin (10mg) were transferred into two separate10mL volumetric flasks, dissolved in methanol and made up the volume to 10mL with the same solvent to give the final strength, i.e. 1000  $\mu$ g/ml. The stock solution was sonicated for 2min.

# Preparation of working standard solution (100µg/mL)

From the above stock solution 1mL each of Sulbactum and Ampicillin were taken, transferred to separate 10mL volumetric flasks and the volume was made up to 10 mL with phosphate buffer.

# Selection of wavelength for analysis of Sulbactum and Ampicillin

Appropriate volume 0.5 ml of standard stock solution of Sulbactum and Ampicillin were

transferred into a 10 ml volumetric flask separately, diluted to a mark with distilled water to give concentration of 5  $\mu$ g/ml. The resulting solution was scanned in the UV range (200–400 nm).

#### Simultaneous Equations Method (Method A)

10µg/mL solutions of Sulbactum and Ampicillin were prepared separately in phosphate buffer (pH 6.8) and the solutions were scanned against blank in the entire UV range to determine the  $\lambda$ max values. Clear peaks were noted. Hence these wavelengths were chosen as the  $\lambda$ max values for each drug respectively. Standard solutions of Sulbactum and Ampicillin in the concentration 10-50µg/mL range of and 10-100µg/mL respectively were prepared in phosphate buffer and the absorbance of these solutions was measured. Calibration curves were plotted to verify the Beer's law and the absorptivity values calculated at the respective wavelengths for both the drugs.

# Absorbance Ratio Method/ Q-Analysis (Method B)

The absorbance ratio method is a modification of the simultaneous equation procedure. It depends on the property that for a substance, which obeys Beer's law at all wavelength, the ratio of absorbance at any two wavelengths is constant value independent of concentration or path length. E.g. two dilutions of the same substance give the same absorbance ratio A1 / A2. In the USP, this ratio is referred to as Q value. In the quantitative assay of two components in admixture by the absorbance ratio method, absorbances are measured at two wavelengths, one being the  $\lambda$  max of one of the components  $(\lambda 2)$  and the other being a wavelength of equal absorptivity of the two components ( $\lambda$ 1), i.e., an iso-absorptive point.A series of standard solutions of Sulbactum and Ampicillin in the concentration range of 10-50µg/mL and 10-

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100µg/mL respectively were prepared in phosphate buffer and the absorbance of these solutions was measured at 292 nm (iso-absorptive point) and 272 nm( $\lambda$ max of Sulbactum). Calibration curves were plotted to verify the Beer's law and the absorptivity values calculated at the respective wavelengths for both the drugs.

#### Assay of tablets by Method A and B

20 commercial tablets of Sulbactum and Ampicillin (Sultacillin) were triturated and powder equivalent to 14.7mg of Sulbactum and 22.0 mg of Ampicillin respectively was weighed and transferred to 10mL volumetric flask, dissolved in methanol, volume adjusted up to the mark with the same solvent and mixed well with the help of a sonicator. The solution was filtered through Whatman filter paper no 40.1mL of the above filtrate was diluted to 10mL with phosphate buffer to obtain a 100µg/mL solution. From this solution an aliquot was taken and made up the volume to 10mL with phosphate buffer expected to contain 14.7 and 22  $\mu$ g/mL of Sulbactum and Ampicillin respectively. The absorbance of the sample solution was measured at 272 nm and 322 nm(Method A), 292 nm and 272nm (Method B) and the data analyzed accordingly using the necessary equations. The analysis procedure was repeated for 6 times with tablet formulations. The result of analysis of tablet formulation is reported in Table 7.2.

#### Validation (Method A&B)

#### Linearity

Appropriate dilutions of working standard solutions for Sulbactum and Ampicillin were prepared in the concentration range of 10- $50\mu$ g/mL and 10- $100\mu$ g/mL, respectively and analyzed as per the developed methods A & B. The results are reported in Table 7.3.

#### **Accuracy and Recovery studies**

To check the accuracy of the proposed method, recovery studies were carried out by standard addition method at three different levels according to ICH guidelines. A series of solutions of Sulbactum and Ampicillin at 80%, 100%, and 120% of the standard preparation in the ratio of the formulation were prepared and checked for accuracy by determining the absorbance values at  $\lambda$ max of 272 nm and 322 nm (Method A) 292 nm and 272nm (method B) respectively. To a fixed concentration of the formulation, varying concentrations of pure drug solutions were added and percentage recoveries calculated. The result of the analysis is given in Table 7.4.

### Precision

Precision studies were performed at three different concentrations in the ratio of the formulation, each concentration prepared three times for Sulbactum and Ampicillin together. The result of the analysis is given in Table 7.4

#### Sensitivity

The sensitivity of measurements of Sulbactum and Ampicillin by the use of the proposed method was estimated in terms of the limit of quantification (LOQ) and limit of detection (LOD). The LOQ and LOD were calculated using equation

 $LOD = 3.3 \times N/B$  and  $LOQ = 10 \times N/B$ ,

where 'N' is standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve.

#### Repeatability

Repeatability was determined by analyzing 20 607

µg/ml concentration of Sulbactum and Ampicillin solutions for six times.

#### Ruggedness

Ruggedness of the proposed method is determined for 20  $\mu$ g/ml concentration of Sulbactum and Ampicillin by analysis of aliquots from a homogenous slot by two analysts using same operational and environmental conditions.

# Determination of Sulbactum and Ampicillin in bulk

Accurately weighed 10 mg of Sulbactum and Ampicillin were transferred into a 100 ml volumetric flask containing 20 ml distilled water separately, and the volume was made up to the mark using the same. Appropriate volume of this solution was transferred to a 10 ml volumetric flask, and the volume was adjusted to the mark using distilled water. The resulting solution was scanned on a spectrophotometer in the UV range 200–400 nm. The concentrations of the drug were calculated from linear regression equations.

#### **RESULTS AND DISCUSSION**

Quality Control is a very important step in the process of drug manufacturing, as it ensures its safety and efficacy. Thus, research on quality control of pharmaceutical products to identify the content of active and the study of physical and chemical characteristics of the drug are essential to ensure the quality of the final product. In order to reduce environmental impacts of their activities on the environment, industries must seek alternatives to reduce, prevent or eliminate chemical residues in their routine processes. Thus, the replacement of analytical methods that employ organic solvents for others that do not employ them is an action may be taken for this purpose<sup>16</sup>. In that context, the UV spectroscopy is a good option because it is a method that does

not use organic solvents and also allows the quantification of compounds.

The present work involves simultaneous estimation of Sulbactum and Ampicillin using UV Spectroscopy. The current trend followed by the industries is developing a methodology which can save sophisticated instruments and chemist's valuable time by which the product analysis can be done very fast, thereby saving the time. This is the reason why people are more attracted towards UV spectroscopy methods, though most of the pharmacopeia still has the UV spectroscopy methods only. Keeping all these points in mind, the current method has been developed and it is very fast and encouraging. The developed method was validated with a holistic approach according to ICH guidelines and details of findings are expressed.

The methods discussed in the present work provided a convenient and accurate way for the analysis of Sulbactum and Ampicillin in bulk and in pharmaceutical dosage form. The absorbance maxima of Sulbactum and Ampicillin was found to be 272 nm and 322 nm respectively for the method A, the absorption maxima of first order derivative spectra was found to be 292 nm for method B.

#### Absorption Maxima and Calibration Curve of Sulbactum and Ampicillin

Appropriate volume 0.5 ml of standard stock solution of Sulbactum and Ampicillin were transferred into a 10 ml volumetric flask separately, diluted to a mark with distilled water to give concentration of 5  $\mu$ g/ml. The resulting solution was scanned in the UV range (200–400 nm). In spectrum Sulbactum and Ampicillin showed absorbance maximum at 272 nm and 322 nm respectively [Figure 7.1]









Figure 7.2 b) Calibration curve of drug Ampicillin

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#### Simultaneous Equations Method (Method A)

 $10\mu$ g/mL solutions of Sulbactum and Ampicillin were prepared separately in phosphate buffer (pH 6.8) and the solutions were scanned against blank in the entire UV range to determine the  $\lambda$ max values. Clear peaks were observed at 272 nm for Sulbactum and 322 nm for Ampicilin.

Hence these wavelengths were chosen as the  $\lambda$ max values for each drug respectively.

Standard solutions of Sulbactum and Ampicillin in the concentration range of  $10-50\mu$ g/mL and  $10-100\mu$ g/mL respectively were prepared in phosphate buffer and the absorbance of these solutions was measured at 272 nm and 322 nm. Calibration curves were plotted to verify the Beer's law and the absorptivity values calculated at the respective wavelengths for both the drugs. Two simultaneous equations as below were formed using these absorptivity values, A (1%, 1cm).

> A1 = 915bCx + 114bCyA2 = 324bCx + 336bCy

Where, Cx and Cy are the concentrations of Sulbactum and Ampicillin measured in gm/100mLin sample solutions. A1 and A2 are the absorbances of mixture at selected wavelengths 272 nm and 322 nm respectively.

# Absorbance Ratio Method/ Q-Analysis (Method B)

The absorbance ratio method is a modification of the simultaneous equation procedure. It depends on the property that for a substance, which obeys Beer's law at all wavelength, the ratio of absorbance at any two wavelengths is constant value independent of concentration or path length. E.g. two dilutions of the same substance give the same absorbance ratio A1 / A2. In the USP, this ratio is referred to as Q value. In the quantitative assay of two components in admixture by the absorbance ratio method, absorbances are measured at two wavelengths, one being the  $\lambda$  max of one of the components  $(\lambda 2)$  and the other being a wavelength of equal absorptivity of the two components ( $\lambda$ 1), i.e., an iso-absorptive point. A series of standard

solutions of Sulbactum and Ampicillin in the concentration range of 10-100µg/mL and 10- $50 \mu g/mL$ respectively were prepared in phosphate buffer and the absorbance of these solutions was measured at 292 nm (isopoint) and 272 nm(λmax absorptive of Sulbactum). Calibration curves were plotted to verify the Beer's law and the absorptivity values calculated at the respective wavelengths for both the drugs. The absorptivity values are reported in Table 7.1.

The concentration of two drugs in mixture was calculated by using the following equations:

 $\begin{array}{l} CX = Qm - QyQx - Qy \times A1aX1 \\ CY = Qm - QxQy - Qx \times A1aY1 \end{array}$ 

Where, A1 and A2 are the absorbances of mixture at 292 nm and 272 nm,ax1 (114.8), ax2 (336.3) and ay1 (915.6), ay2 (324.6) are A (1%, 1 cm) of Sulbactum and Ampicillin at 292 nm and 272 nm respectively,

Qm = A2A1, Qx = ax2ax1 and Qy = ay2ay1

Table 7.1: Absorption values (A 1%, 1 cm) of Sulbactum and Ampicillin for methods A & B

Absorptivity, A (1%, 1cm)						
	Metl	Method B				
Con. (µg/ml)	Sulbactum	Ampicillin	Sulbactum		Ampicillin	
		А	bsorbance's			
	272nm	322nm	292nm	272nm	292nm	322nm
10	0.42	0.11	0.37	0.42	0.02	0.11
20	0.63	0.20	0.43	0.63	0.06	0.2
30	0.81	N/A	0.57	0.81	N/A	N/A
40	1.1	0.32	0.68	1.1	0.12	0.32
50	1.22	N/A	0.88	1.22	N/A	N/A
60	N/A	0.45	N/A	N/A	0.21	0.45
80	N/A	0.62	N/A	N/A	0.33	0.62
100	N/A	0.78	N/A	N/A	0.42	0.78
Mean	0.836	0.41	0.586	0.836	0.1933	0.41

Table 7.2: Regression analysis of calibration curves and summary of validation parameters for Methods A & B

No	Parameters	Drug	Method A		Method B	
			272nm	322nm	292nm	272nm
1	Beer's law limit	Sulbactum	N	N/A		N/A
	$(\mu g m l^{-1})$	Ampicillin	N	/A	10-100	N/A
2	Molar	Sulbactum	30614	11223	28012	30614
	absorptivity	Ampicillin	2666	9082	5010	2266
	$(1 \text{ mol}^{-1} \text{ cm}^{-1})$					
3	Saldell's	Sulbactum	0.01	0.029	0.046	0.01
	sensitivity	Ampicillin				
	$(\mu g/cm^2/0.001)$		0.083 0.026		0.05	0.91
4	Intercept(c)	Sulbactum	0.0418	0.004	0.004	0.041
		Ampicillin	0.018	0.0071	0.018	0.018
5	Slope (m)	Sulbactum	0.0895	0.034	0.020	0.089
	Correlation	Ampicillin	0.09	0.0365	0.019	0.009
6		Sulbactum	0.9992	0.999	0.999	0.999
	Coefficient (r <sup>2</sup> )	Ampicillin	0.998	0.9993	0.999	0.9989

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#### Validation (Method A&B)

#### Linearity

Appropriate dilutions of working standard solutions for Sulbactum and Ampicillin were prepared and observed Linearity for the method A and method B in the concentration range of  $10-50\mu$ g/mL and  $10-100\mu$ g/mL, respectively and analyzed as per the developed methods A & B.

The results are reported in Table 7.3 The assays of the both methods were found to be within the range of 98-102%.

#### **Accuracy and Recovery studies**

The validations of proposed methods were further confirmed by accuracy and recovery studies by standard addition method at both the different levels according to ICH guidelines. A series of solutions of Sulbactum and Ampicillin at 80%, 100%, and 120% of the standard preparation in the ratio of the formulation were prepared and checked for accuracy by determining the absorbance values at  $\lambda max$  of 272 nm and 322 nm (Method A) 292 nm and 272nm (method B) respectively. To a fixed concentration of the formulation, varying concentrations of pure drug solutions were added and percentage recoveries calculated. The %recovery values vary from 98-102% as shown in the table 7.3. The result of the analysis is given in Table 7.3.

Level of Recovery (%)	Recovery(%) ± SD					
	Method	l A	Method B			
	Sulbactum Ampicillin		Sulbactum	Ampicillin		
80	98.8±0.17	99±0.13	98±0.5	98.9±1.3		
100	98.9±0.18	98.7±0.26	98.7±0.15	99.7±0.19		
120	98.1±0.42	98.3±0.45	98.3±0.28	98.3±1.01		

#### Table 7.3: Results for recovery studies

#### Precision

Precision studies were performed at three different concentrations in the ratio of the formulation, each concentration prepared three times for Sulbactum and Ampicillin together. The result of the analysis is given in Table 7.4. The precision of the method was expressed in terms of % relative standard deviation (% RSD). The % RSD values were found to be less than 2 for intraday and inter day precision, the precision results showed good reproducibility.

Table '	7.4:	Results	for	precision	studies
1 auto	/	Results	101	precision	studies

No.	Conc (µg/mL)		*Assay(%) ± SD			
			Method A		Method B	
	Sulbactum Ampicillin		Sulbactum	Ampicillin	Sulbactum	Ampicillin
1	10	20	98.7±0.23	98.93±0.12	98.26±0.64	99.3±0.41
2	20	40	98.73±0.23	99.26±0.23	99.2±0.69	98.7±0.26
3	30	60	98.83±0.57	98.38±0.68	98.2±0.69	98.67±0.29

#### Sensitivity

The sensitivity of measurements of Sulbactum

and Ampicillin by the use of the proposed method was estimated in terms of the limit of quantification (LOQ) and limit of detection (LOD). The LOQ and LOD were calculated using equation

 $LOD = 3.3 \times N/B$  and  $LOQ = 10 \times N/B$ ,

where 'N' is standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve.

### Repeatability

Repeatability was determined by analyzing 20 µg/ml concentration of Sulbactum and Ampicillin solutions for six times.

### Ruggedness

Ruggedness of the proposed method is determined for 20  $\mu$ g/ml concentration of Sulbactum and Ampicillin by analysis of aliquots from a homogenous slot by two analysts using same operational and environmental conditions.-

# SUMMARY AND CONCLUSION

Two new, simple, sensitive and economical UV spectrophotometric methods were developed for the simultaneous analysis of Sulbactum and Ampicillin in bulk and in pharmaceutical formulations (SULTAMICILLIN).

Analytic method development and validation are continuous interconnected and activities conducted throughout the drug development process. The practice of validation verifies that a given method measures a parameter as intended and establishes the performance limits of the measurement. Although apparently contradictory, validated methods produce results within known uncertainties. These results are crucial to continuing drug development, as they define the emerging knowledge base supporting the product.

The time and effort that are put into developing scientifically-sound, robust, and transferrable analytic methods should be aligned with the drug development stage. The re-sources that are expended on method validation must be constantly balanced with regulatory requirements and the probability for product commercialization.

Method I is based on solving simultaneous equation. Sulbactum and Ampicillin show absorbance maximums at 272 and 332 nm respectively, so absorbance was measured at the same wave lengths for the estimation of Sulbactum and Ampicillin.

Method II is based on determination of Q-value. Both drugs (Sulbactum and Ampicillin) obey the Beer Lambert's law in the concentration range of 5-30 µg/mL and the correlation coefficient values were found to be 0.9994 and 0.999 respectively Methods are validated according to ICH guidelines and can be adopted for the routine analysis of Sulbactum and Ampicillin in tablet pure and dosage form of SULTAMICILLIN. The developed methods were validated and from the statistical data, it was found that the methods were linear, accurate and precise and can be successfully applied for the analysis of pharmaceutical formulations without interference of excipients. The proposed method is simple, selective and sensitive.

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