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SPECTROPHOTOMETRIC METHOD DEVELOPED FOR THE ESTIMATION OF FLUCLOXACILIN IN BULK AND DOSAGE FORM USING UV-VIS SPECTROPHOTOMETRIC METHOD

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ABSTRACT

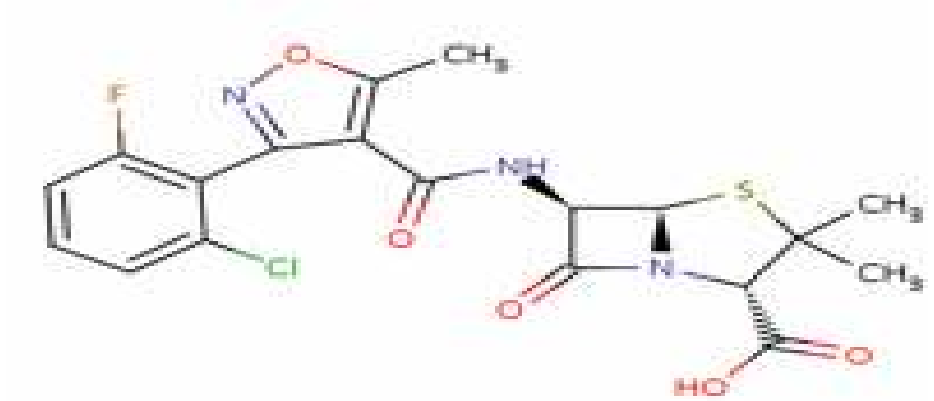
The present study describes a simple, accurate, precise and cost effective UV-VIS Spectrophotometric method for the estimation of FLUCLOXACILLIN, is a penicillin beta-lactam antibiotic, in bulk and pharmaceutical dosage form. The solvent used was double distilled water and the λmax or the absorption maxima of the drug was found to be 219nm. A linear response was observed in the range of 2-10µg/ml with a regression coefficient of 0.998. The method was then validated for different parameters as per the ICH [2](International Conference for Harmonization) guidelines. This method can be used for the determination of FLUCLOXACILLIN in quality control of formulation without interference of the excipients.

KEYWORDS

FLUCLOXACILLIN, λmax, ICH, UV-VIS spectroscopy, Beta-lactam antibiotic.

INTRODUCTION

FLUCLOXACILLIN, chemically[1] (2S,5R,6R)-6-[[3-(2-chloro-6-fluorophenyl)-5-methyl1,2-oxazole-4-carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid [1].



FLUCLOXACILLIN is a penicillin beta-lactam antibiotic used in the treatment of bacterial infections. FLUCLOXACILLIN chemical formula is $C_{19}H_{17}ClFN_3O_5S$ [1]. FLUCLOXACILLIN is usually used to treat the gram-positive, organisms. FLUCLOXACILLIN The name "penicillin" can either refer to several variants of penicillin available, or to the group of antibiotics derived from the penicillins. Flucloxacillin has *in vitro* activity against gram-positive and gram-negative aerobic and anaerobic bacteria. The bactericidal activity of Flucloxacillin results from the inhibition of cell wall synthesis and is mediated through flucloxacillin binding to penicillin binding proteins (PBPs)[1]. FLUCLOXACILLIN is stable against hydrolysis by a variety of beta-lactamases, including penicillinases, and cephalosporinases and extended spectrum beta-lactamases. By binding to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall, flucloxacillin inhibits the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that flucloxacillin interferes with an autolysin inhibitor.. Literature survey revealed that FLUCLOXACILLIN was estimated either individually or in presence of amoxicillin trihydrate by using RP-HPLC method [4, 5] and no work has been done in UV-VIS Spectrophotometry.

Materials and Methods*:* The instrument used for the study was an UV-VIS double beam spectrophotometer (Model T60, Analytical Technologies Limited) with 1cm matched pair quartz cells. The solvent used was double distilled water.

METHOD DEVELOPMENT [2]

Solubility Test*:* Solubility test for the drug FLUCLOXACILLIN was performed by using various solvents. The solvents include Distilled water, Methanol, Ethanol, Acetonitrile, 0.1 N Hydrochloric Acid (HCl), 0.1 N Sodium Hydroxide (NaOH) and Chloroform. However, Distilled water was chosen as a solvent for developing the method.

Determination of λmax

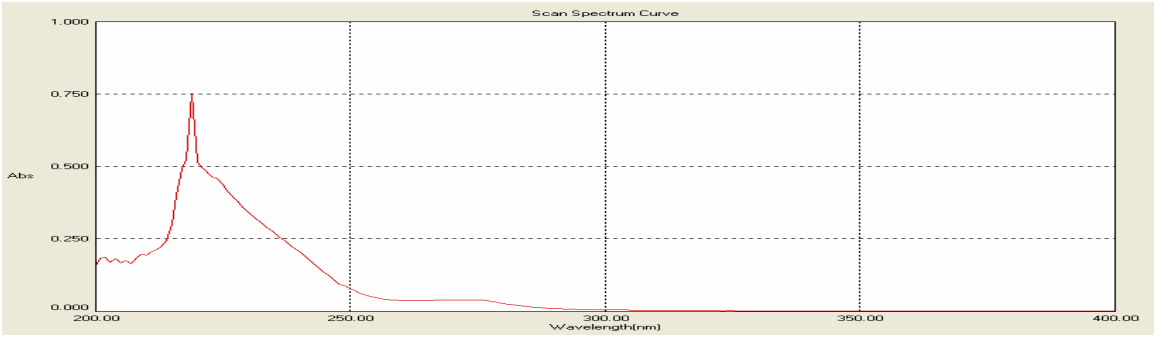
Preparation of Stock Solution: Standard stock solution of FLUCLOXACILLIN was prepared by dissolving 10mg of FLUCLOXACILLIN sodium in 10ml of 0.1N NaOH to produce a concentration of 1000µg/ml. 1ml of this stock solution was taken and then diluted up to 10ml by using 0.1N NaOH to produce a concentration of 100µg/ml which is the standard stock solution.

Preparation of Working Standard Solution: From the above stock solution, 1ml was

pippetted into a 10ml volumetric flask and the volume was made up to the mark with 0.1N NaOH to prepare a concentration of 10µg/ml. Then the sample was scanned in UV-VIS Spectrophotometer in the range 400-200nm using 0.1N NaOH as a blank and the wavelength corresponding to maximum absorbance (λmax) was found to be 219nm (fig. 1).

Figures:

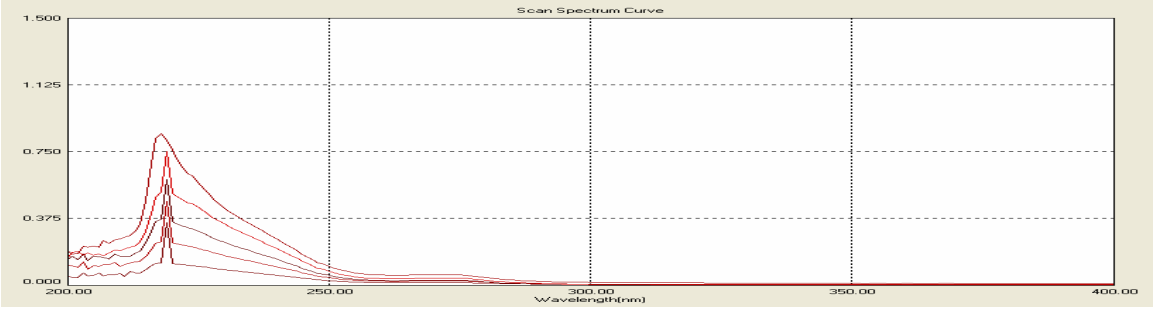
Determination of λ_{max}:



λmax of FLUCLOXACILIN showing at 219nm

(fig. no. 1)

Preparation of Calibration Curve*:* 1ml of the 100µg/ml solution was diluted to 10ml by using 0.1N NaOH to produce 10µg/ml solution. Then this 10µg/ml was further diluted with 0.1N NaOH to produce a concentration of 2µg/ml, 4µg/ml, 6µg/ml and 8µg/ml and the construction of calibration curve was done by taking the above prepared solutions of different concentration ranging from 2-10µg/ml. Then, the calibration curve was plotted by taking concentration on x-axis and absorbance on y-axis (in fig.2). The curve showed linearity in the concentration range of 2-10µg/ml. The correlation coefficient (r^2) was found to be 0.998.



Showing λmax of FLUCLOXACILLIN at conc. 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml and 10µg/ml at a wave length of 219nm

(fig. no. 2)

Assay of FLUCLOXACILLIN tablet [3]

A quantity of powder equivalent to 50mg of FLUCLOXACILLIN was taken in a 50ml volumetric flask and it was dissolved and diluted upto the mark with 0.1N NaOH. The resultant solution was ultrasonicated for 5 minutes. The solution was then filtered using Whatmann filter paper No.41. From the filtrate, appropriate dilutions were made in 0.1N NaOH to obtain the desired concentration (10µg/ml). This solution was then analysed in UV and the result was indicated by % recovery given in table 1.

METHOD VALIDATION [2]***:***

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics.

The method was validated for different parameters like Linearity, Accuracy, Precision, Specificity, Robustness, Ruggedness, Limit of Detection (LOD) and Limit of Quantification (LOQ).

Linearity: *Various aliquots were prepared form the stock solution (100µg/ml) ranging from 2-10µg/ml.* The samples were scanned in UV-VIS Spectrophotometer using 0.1N NaOH as blank. It was found that the selected drug shows linearity between the 2-10µg/ml (table 2&1).

VALIDATION: Table for Linearity:

Table No. 2

Linearity Table of FLUCLOXACILLIN Sodium in Working Standard

Concentration (µg/ml)	Absorbance
0	0
2	0.223
4	0.412
6	0.593
8	0.763
10	0.953

Accuracy: The accuracy of the method was determined by preparing solutions of different concentrations that is 80%, 100% and 120% in which the amount of marketed formulation was kept constant (10mg) and the amount of pure drug was varied that is 8mg, 10mg and 12mg for 80%, 100% and 120% respectively. The solutions were prepared in triplicates and the accuracy was indicated by % recovery (table 4).

Table no. 4

Accuracy Readings of FLUCLOXACILLIN Sodium

OBSERVATION / RESULTS						
No. of preparations	Concentration (µg/lml)		% Recovery	Statistical Results		
	Formulation	Pure Drug		Mean	SD	%RSD
S_1 : 80 %	10	8	99.8	100.16	1.484363	1.48
S_2 : 80 %	10	8	101.8			
S_3 : 80 %	10	8	98.9			
S_4 : 100 %	10	10	100.1	100.56	1.078579	1.07
S_5 : 100 %	10	10	101.8			
S_6 : 100 %	10	10	99.8			
S_7 : 120 %	10	12	101.8	100.06	1.553491	1.55
S_8 : 120 %	10	12	98.8			
S_9 : 120 %	10	12	99.6			

Precision: Precision of the method was demonstrated by intraday and interday variation studies. In intraday variation study, 9 different solutions of same concentration that is 8µg/ml were prepared and analysed three times in a day i.e. morning, afternoon and evening and the absorbances were noted. The result was indicated by % RSD (table no.5, & table no.6). In the interday variation study, solutions of same concentration 8µg/ml were prepared and analysed three times for three consecutive days and the absorbances were noted. The result was indicated by % RSD (table no.7).

Table no. 7

Inter-assay Precision

Concentrations (µg/ml)	%RSD			Average %RSD
	Day 1	Day2	Day3	
8	0.14%	0.19%	0.19%	0.173%

Specificity: 10mg of FLUCLOXACILLIN was spiked with 50% (5mg), 100% (10mg), and 150% (15mg) of excipient mix (Magnesium Stearate) and the sample was analysed for % recovery of FLUCLOXACILLIN (table no.8).

Test for Specificity:

Table no. 8

Sample No.	Excipient Conc.(%)	Flucloxacillin Input(mg)	Flucloxacillin Recovered (mg)	Flucloxacillin Recovered (%)	Mean Recovered (%)	S.D.	%R.S.D.
1	100%	10	9.97	99.7%			
2	50%	10	9.96	99.6%	99.56%	0.152753	0.153428
3	150%	10	9.94	99.4%			

Test for Specificity showing no effect of excipient.

Robustness: Robustness of the method was determined by carrying out the analysis at two different temperatures i.e. at room temperature and at 18ºc. The respective absorbances of 8µg/ml were noted and the result was indicated by % RSD (tableno.9).

Ruggedness & Robustness

Table No. 9

Results Showing Robustness and Ruggedness of Method for FLUCLOXACILLIN

Analyst-1			Analyst-2		
Conc. (µg/ml)	Abs.	Statistical Analysis	Conc. (µg/ml)	Abs.	Statistical Analysis
8	0.762	Mean = 0.7616	8	0.760	Mean = 0.761
8	0.761	SD = 0.000548	8	0.763	SD = 0.001225
8	0.762		8	0.761	
8	0.762	%RSD = 0.07%	8	0.760	%RSD = 0.16%
8	0.761		8	0.761	
Room Temperature			**Temp. 18°C**		
Conc. (µg/ml)	Abs.	Statistical Analysis	Conc. (µg/ml)	Abs.	Statistical Analysis
8	0.763	Mean = 0.7618	8	0.761	Mean= 0.7612
8	0.762	SD = 0.001304	8	0.762	SD = 0.001304
8	0.763		8	0.762	
8	0.761	%RSD = 0.17%	8	0.759	%RSD = 0.17%
8	0.760		8	0.762	

Limit of Detection (LOD)

The LOD for FLUCLOXACILLIN Sodium was found to be 0.15µg/ml.

Limit of Quantification (LOQ)

The LOQ for FLUCLOXACILLIN Sodium was found to be 0.45µg/ml.

Ruggedness: Ruggedness of the method was determined by carrying out the analysis by two different analysts and the respective absorbances of 8µg/ml were noted. The result was indicated by % RSD (table no.9).

Limit of Detection (LOD): The limit of detection (LOD) was determined by preparing solutions of different concentrations ranging from 0.1-0.5µg/ml. The detection limit of an individual analytical procedure is the lowest

amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value (table no.1).

Limit of Quantification*:* The LOQ is the concentration that can be quantification reliably with a specified level of accuracy and precision. The LOQ was calculated using the formula involving standard deviation of response and slope of calibration curve (table no.1).

TABLE No.1

SUMMARY OF VALIDATION

PARAMETER	RESULT
Linearity indicated by correlation coefficient	0.998
Precision indicated by %RSD	0.147%
Accuracy indicated by % recovery	98.8%-101.8%
Specificity indicated by % recovery	99.56%
Limit of Detection	0.15µg/ml
Limit of Quantification	0.45µg/ml
Range	2-10µg/ml
Linear regression equation	y = 0.093x + 0.021
Robustness & Ruggedness indicated by %RSD	0.1425%
Assay indicated by % recovery	101.8%

RESULTS AND DISCUSSION

The developed method was found to be precise as the %RSD values for intra-day and inter-day were found to be less than 2%. Good recoveries (99.67% to 101.8%) of the drug were obtained at each added concentration, indicating that the method was accurate. The method was also found to be specific indicated by the % recoveries ranging from 98.8% to 101.8%. The LOD and LOQ were found to be in sub-microgram level indicating the sensitivity of the method. The method was also found to be robust and rugged as indicated by the %RSD values which are less than 2%. The results of Assay show that the amount of drug was in good agreement with the label claim of the formulation as indicated by % recovery (101.8%). Summary of validation parameters of proposed spectrophotometric method is shown in table 1.

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Table No. 3

Optical characteristics

Beer's Law limit (µg/mL)	2-10µg/ml
Molar extinction coefficient (1 mole-1 c.m-1)	953
Correlation coefficient	0.998s
Regression equation (Y*)	y = 0.093x + 0.021
Slope (a)	0.093x
Intercept (b)	0.021

Precision:

Table no. 5

Precision Results Showing Repeatability of FLUCLOXACILLIN Sodium

Concentrations (µg/ml)	Absorbance	Statistical Analysis
8	0.761	
8	0.763	
8	0.762	
8	0.763	Mean = 0.762125
8	0.763	SD = 0.000835
8	0.762	%RSD = 0.10%
8	0.761	
8	0.762	

Table no. 6

Intra-assay Precision:

Concentrations (µg/ml)	Absorbance 1	Absorbance 2	Absorbance 3	Average %RSD
8	0.763	0.762	0.763	
8	0.762	0.762	0.761	
8	0.762	0.761	0.763	
8	0.759	0.761	0.763	
8	0.758	0.763	0.762	
8	0.761	0.758	0.761	
8	0.762	0.763	0.762	
8	0.763	0.762	0.761	
8	0.762	0.761	0.762	
%RSD	**0.22%**	**0.19%**	**0.11%**	**0.17%**

Preparation of Calibration Curve:

Calibration Curve of FLUCLOXACILLIN

conc.	Abs
0	0
2	0.223
4	0.412
6	0.593
8	0.763
10	0.953

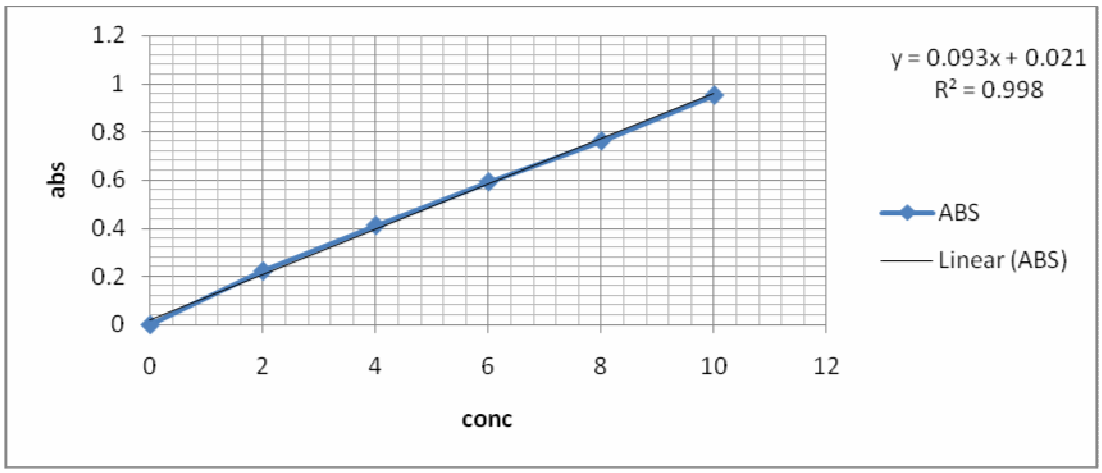

Fig. No. 3

Calibration Curve of Flucloxacillin

CONCLUSION

All the above factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive, robust and cost effective and can be applied successfully for the estimation of FLUCLOXACILLIN in bulk and pharmaceutical formulation.

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