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UV Visible Spectrophotometric Estimation of Antibiotic Drugs

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ABSTRACT

The main aim of current study was to develop UV Visible spectrophotometric method for the quantitative determination of Flucloxacillin and Ceftriaxone in bulk and pharmaceutical formulations. Flucloxacillin was a broad spectrum beta -lactam antibiotic, belonging to the isoxazolyl family of penicillin's. Ceftriaxone was a semi synthetic, broad-spectrum and third generation cephalosporin antibiotic for intravenous or intramuscular administration. The solvents used in this method was chloroform, double distilled water (1:1) in presence of phosphate buffer of pH 7.4. Linearity test solutions for the assay method were prepared in the range of 2-12 µg/ mL and 4-16 µg/mL respectively for determination of flucloxacillin and ceftriaxone which obeyed Beer's law and found to linear. From the experimental results reveal that absorption maximum of 270 nm for flucloxacillin and 290 nm for ceftriaxone were found. Validation of the developed method for two drugs was carried out as per ICH requirements.

Keywords: Flucloxacillin, Ceftrixone, UV visible spectrophotometry, Beer's law, validation[2].

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INTRODUCTION

Flucloxacillin is a broad spectrum beta -lactam antibiotic, belonging to the isoxazolyl family of penicillins. It is effective treating drug to cure diseases caused by pencillinase –resistant staphylococci. It is a crystalline white powder and freely soluble in methanol, ethanol and water. Chemically flucloxacillin is (2*S*, 5R, 6R)-6-({[3-(2-chloro-6-fluorophenyl)-5-methylisoxazole -4-yl] carbonyl} amino)-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid[1]. The chemical formula of this compound is $C_{19}H_{17}ClFN_3O_5S$[1]. It has a calculated molecular weight of 453.87 g/mol. Ceftriaxone is a semi synthetic, broad-spectrum and third generation cephalosporin antibiotic for intravenous or intramuscular administration.It is used to treat a wide variety of bacterial infections. It is a white to yellowish crystalline powder which is readily soluble in water. The color of ceftriaxone sodium solutions ranges from light yellow to amber, Ceftriaxone sodium is (6R, 7R)-7-[2-(2-Amino-4-thiazolyl) glyoxylamido]-8-oxo-3-[[(1,2,5,6-tetrahydro-2-methyl-5,6-dioxo-as-triazin-3-yl)thio]methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 72-(Z)-(O-methyloxime), disodium salt, sesquaterhydrate. The chemical formula of ceftriaxone sodium is $C_{18}H_{16}N_8Na_2O_7S_3 3.5H_2O$. It has a calculated molecular weight of 661.60 g/mol.The molecular structures of Flucloxacillin and Ceftriaxone were shown in fig. 1&2.

As per the literature survey reveals the drugs are estimated quantitative by several techniques including, A modified spectrophotometric method was developed for the determination of some ß-lactam antibiotics including Fluclox and Diclox with pyrocatecholviolet as chromogenic reagent[3] and potassium periodate[4].Diclox was determined spectrophotometrically in a binary mixture with amoxicillin[5]. High performance thin-layerchromatography was developed for determination of chloramphenicol, ampicillin, benzyl penicillin, flucloxacillin and erythromycin in cow's milk[6]. Flucloxacillin drug has been estimated quantitatively through spectrophotometry by some researchers including M.Fiorentino etal[7] using Ultra purified water as solvent, Bromocresol green and chloroform as solvent by R.Vijayalakshmi etal[8],Chloroanilic acid and DDQ as solvent by Refat MS and Eldidamony etal[9], 2,4-Dinitrophenol and 3,5-Dinitro salicylic acid as reagent by Magda Y El-mammli etal[10],Doubledistilled water and NaOH as solvent by Sudasatya dey and Ratnakar etal [11] . During the literature review on ceftriaxone few authors reported its estimation with different reagents and solvents including Folin –Ciocalteu as reagent by N.M.Patel etal[12] , 5% Para dimethyl Amino Benzaldehyde as a reagent by S.A.Thangathurai etal[13], NaOH – Variamine Blue as a reagent by B.Narayana etal[14], Ferric Chloride as a reagent by W.Quasim etal[15] and 4- Dimethyl amino benzaldehyde by F.M.A.Rind etal[16] .comparisons of the proposed

method with other existing methods for the assay of Flucloxacillin and Ceftriaxone in pharmaceutical formulations are shown in table 4-5 .

All the reported methods are not precise, simple, accurate and less expensive. Therefore, an attempt was made to develop a low cost, precise, economical, sensitive, routine and accurate spectrophotometric method for the quantification of Flucloxacillin and Ceftriaxone in bulk drugs and pharmaceutical formulations.

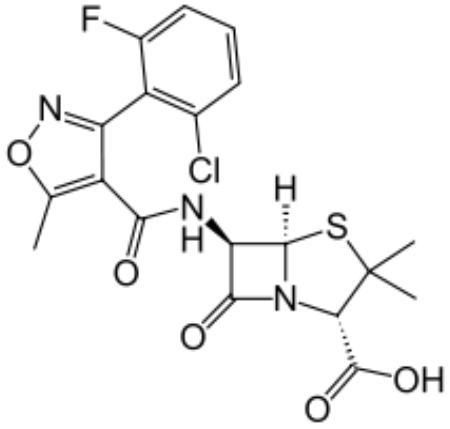

Figure1: Structure of Flucloxacillin

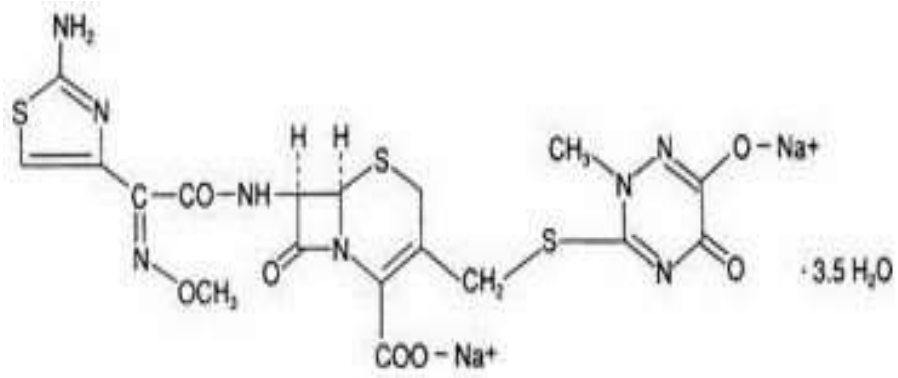

Figure 2: Structure of Ceftriaxone

MATERIALS AND METHOD

Instruments and Apparatus :

The spectrophotometric measurements for both the drugs were carried out by using UV-visible double beam spectrophotometer of model 1700 shimadzu with 1 cm matched quartz cell , spectral band width is1 nm and is supported by UV win 5.0 software.

Reagents and Chemicals:

All chemicals used in this analysis were AR grade. Chloroform and double distilled water in presence of phosphate buffer of pH 7.4 were used as solvents and diluents throughout the analysis. Pharmaceutical formulation of flucloxacillin and Ceftriaxone was supplied by M/S Sun

pharmaceuticals, Mumbai (Maharastra) ,Chloroform, Double distilled water and phosphate buffer of pH 7.4 was purchased from M/S Merck India Ltd, Mumbai (Maharashtra). These drugs were found commercially in the form of tablets namely Flox500 mg, Actinase 500mg, for flucloxacillin Auxil 200 mg, Belox 200 mg for Ceftriaxone.

Selection of Solvent:

The buffer selected for Spectrophotometric analysis of flucloxacillin and ceftriaxone were phosphate buffer of pH 7.4, chloroform and double distilled water were used as solvents throughout the analysis.

Selection of Method and Wave Length:

Analytical wavelength of flucloxacillin and ceftriaxone are 270 nm and 290 nm respectively was used for the proposed method. The intercept of calibration line was determined by linear regression Analysis.

Preparation of Standard Solutions of Flucloxacillin and Ceftriaxone:

The 100 mg of standard (pure) drug of flucloxacillin and ceftriaxone were weighed accurately and then transferred into 100 ml volumetric flasks to get 1000 µg/ mL of concentration for the both drugs by dissolved in chloroform solvent. The stock solutions of both the drugs are diluted with double distilled water to get 2, 4,6,8,10,12,14,16 and 18 µg/mL were taken in a ten 10 ml volumetric flasks separately and make up volume with double distilled water. To each flask 2mL of phosphate buffer of pH 7.4 solution is added. The calibration curve was plotted in the concentration range of 2-12 µg/ mL and 4-16µg/mL for determination of flucloxacillin and ceftriaxone respectively in chloroform as a blank. UV scan range of lambda max (λmax) 200 nm to 800 nm was selected to determine maximum absorbance for both the drugs. In this method the wavelength corresponding to maximum absorbance was found at 270 nm for flucloxacillin and 290 nm for ceftriaxone .

Preparation of Sample Solutions of Flucloxacillin and Ceftriaxone:

For the analysis of flucloxacillin two commercial brands namely Flox (500 mg) Actinase (500mg) tablets and Auxil (200mg), Belox (200mg) tablets for ceftriaxone were procured from Apollo pharmacy , Chennai (TN).Ten tablets of each brand of both drugs weighed accurately and powdered .100 mg of both drugs in powdered form dissolved in 40 ml of chloroform separately and sonicated for few minutes and filtered by using whatmann filter paper No.42.The filtrate formed is diluted with double distilled water to get10 µg/mL concentrations of both drugs.To each flask 2mL of phosphate buffer of pH 7.4 solution is added, and the absorbance of flucloxacillin and ceftriaxone measured at 270 nm and 290 nm respectively.

Determination of λMax :

UV scan range of 200 nm to 800 nm was selected to determine maximum absorbance by using 10 µg/ml solution of both drugs separately the wavelength corresponding to maximum absorbance was found at 270 nm and 290 nm for flucloxacillin and ceftriaxone respectively. The spectrophotometric spectrums are shown in fig.3 & 4.

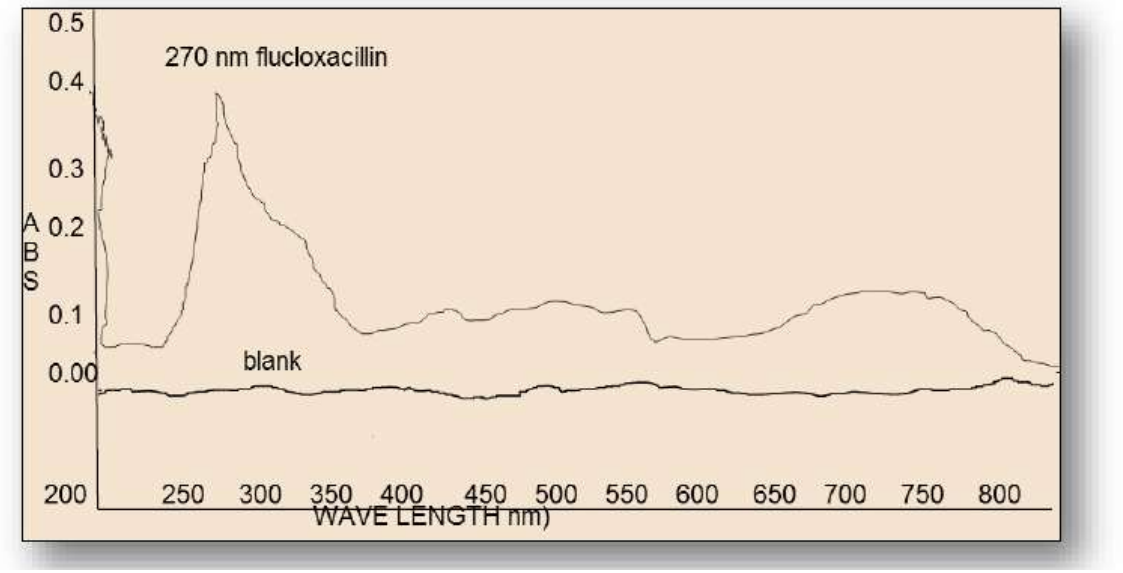

Figure: 3 UV Visible Spectrum of Flucloxacillin

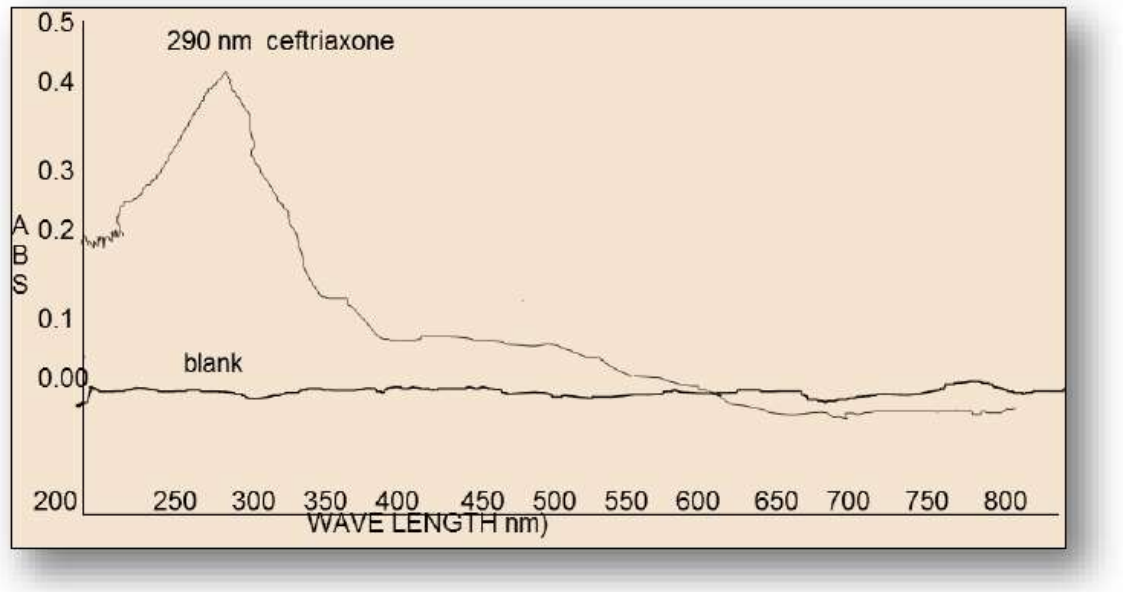

Figure: 4 UV Visible Spectrum of Ceftriaxone

Preparation of Calibration Curve:

On the basis of experimental results, calibration curve were plotted and shown in fig.5& 6 in the concentration range of 2-12 µg/ mL and 4-16µg/ mL of eight standard solutions of flucloxacillin

and ceftriaxone respectively in chloroform as a blank . UV scan range of 200 nm to 800 nm was selected to determine maximum absorbance for both drugs. In this method the wavelength corresponding to maximum absorbance was found to be at 270 nm and 290 nm for flucloxacillin and ceftriaxone respectively.

Validation of Method:

The spectrophotometric analysis of flucloxacillin and ceftriaxone are validated as per the directions of International conference on Harmonisation to determine linearity, precision, accuracy, LOD and LOQ of the method[2] .

Linearity and Range:

Standard stock solutions of flucloxacillin and ceftriaxone in appropriate dilution were assayed as per the proposed method. According to Beer's –Lambert's law the concentration range was found to be 2-12 µg/ mL for flucloxacillin and 4-16 µg/ mL for ceftriaxone and calibration plots in fig.5 and fig. 6 is linear in the given range.

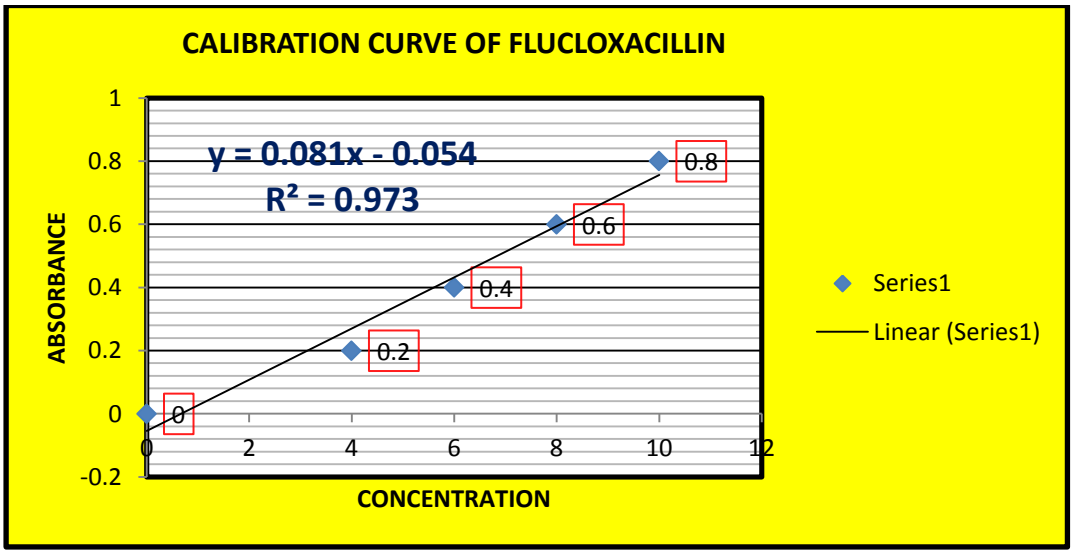

Figure5: Calibration Curve of Flucloxacillin

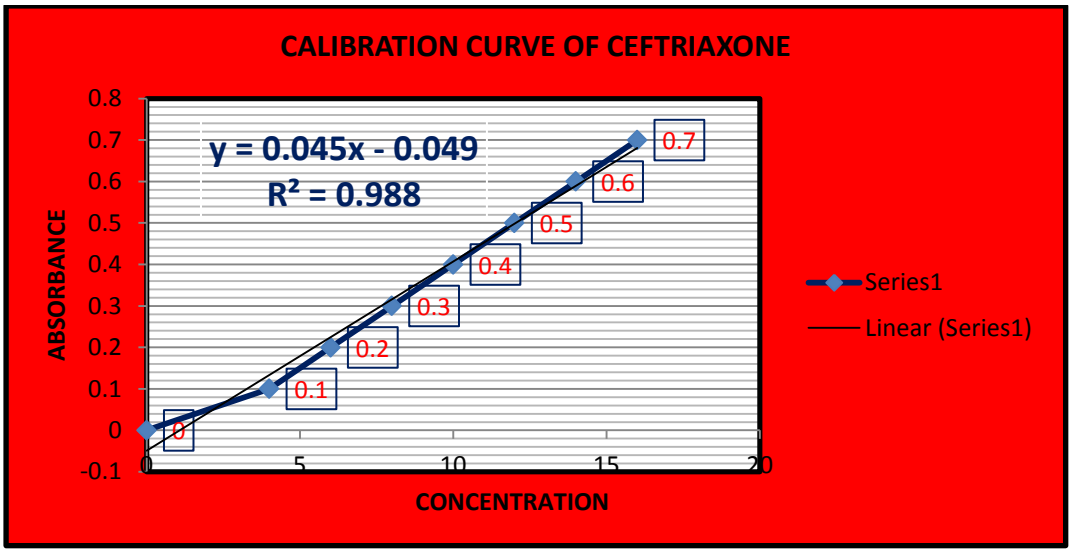

Figure 6: Calibration Curve of Ceftriaxone

Precision

The precision of the proposed method of flucloxacillin and ceftriaxone was estimated by using drug concentrations of both drugs were analyzed six times in a day (intra-day precision) and for six continuous days (inter-day precision). The analytical data for both drugs have shown in the table-1

Table1: Determination of Accuracy and Precision of Flucloxacillin and Ceftriaxone

S.NO	Name Of The Sample	Labeled Amount(mg/capsule)	Amount found* (mg)	Precision	
				Inter day	Intraday
1	Flox	500	499.20	0.0078	0.0062
2	Actinase	500	499.99	0.0089	0.0069
3	Auxil	200	199.88	0.0094	0.0082
4	Belox	200	199.90	0.0096	0.0098

(*average of six determinations)

Accuracy:

The Accuracy of the proposed method of flucloxacillin and ceftriaxone was estimated by using standard addition method. This process is carried out by adding different amounts namely 80% ,100% and 120% of the pure sample for both drugs to the pre-analyzed formulation. Accuracy data for both drugs shown in the table-1.

LOD and LOQ[6]:

LOD is Limit of Detection and LOQ is Limit of Quantitation. The LOD and LOQ of flucloxacillin and ceftriaxone were determined by using standard deviation of the response and slope approach as per the directions of International Conference on Harmonization (ICH) guidelines [2] .The limits of detection (LOD) is calculated by using the equation $LOD = \frac{3s}{k}$

Where, S = intercept of the standard deviation K = The slope of the calibration curve (mean) . The limits of quantitation (LOQ), is calculated by using the equation LOQ $= \frac{10\,S}{K}$ Where, S = intercept of the standard deviation K = The slope of the calibration curve (mean).

Recovery Studies of Flucloxacillin and Ceftriaxone

Recovery of flucloxacillin and ceftriaxone were performed to know the accuracy of the proposed method. This process is done by adding a known quantity of pure drug to a pre-analyzed sample. The result of analysis of both drugs and recovery studies are notified in the table: 2.

Table 2: Recovery Analysis of Marketed Formulations of Flucloxacillin and Ceftriaxone

S.NO	Name of the sample	Label claim (mg)	% Level	Amount found (mg)	% Recovery
			Flucloxacillin		
1	Flox	500	80	499.20	99.840
2	Actinase	500	100	499.99	99.998
			Ceftriaxone		
3	Auxil	200	120	199.88	99.94
4	Belox	200	80	199.99	99.995

RESULTS AND DISCUSSION

The U.V Spectrum of standard stock solutions of flucloxacillin shows absorption maximum at 270 nm and ceftriaxone shows absorption maximum at 290 nm , then calibration curve is obtained by plotting a graph of absorbance verses concentration , the Beer –lamberts' law was verified from the data of calibration curve for both drugs under investigation. The calibration plots for the two drugs have shown in from fig.5 &6. The linearity was observed between 2-12 µg/ mL for flucloxacillin and 4-16 µg/ mL for ceftriaxone. The graph of both drugs shows a straight line with correlation coefficient of 0.9730 for flucloxacillin and 0 .9880 for ceftriaxone.The validated optical, statistical parameters, LOD and LOQ data for Flucloxacillin and Ceftriaxone is depicted in table. 3.

Table 3 : Optical Parameters of Flucloxacillin and Ceftriaxone

S.no	Parameter	Flucloxacillin	Ceftriaxone
1	λMax (nm)	270nm	290nm
2	Beer's Law Limit (µg/ mL)	2-12	4-16
3	Correlation Coefficient(r^2)	0.9730	0.9880
4	Regression Equation (Y= a+bc)	Y=0.081X-0.054	Y=0.045X-0.049
5	Intercept (a)	0.0540	0.0490
6	Slope (c)	0.0810	0.0450
7	SD	3.3166	3.8944
8	Mean	7	10
9	Variance	11	15.1666

10	LOD (%)	0.122	0.259
11	LOQ(%)	0.409	0.865

The assay method for two drugs was validated by accuracy and precision of the proposed method shown in table 1. The % recovery of 99.75 – 100.2 shows accuracy of the proposed method. The result of analysis of both drugs and recovery studies are incorporated in table: 2

Table 4 : Comparisons of the proposed method with other existing methods for the assay of flucloxacillin in pharmaceutical formulations.

S.no	Reagents/Solvent	λMax	Beer's law limits µg mL^{-1}	Correlation coefficient (R^2)	Reference
1	Ultra-purified water	274 nm	50.0 to 100.0	0.9998.	7
2	Bromocresol green, Chloroform	433 nm	0.5-2.5	0.9996	8
3	Chloranilic acid-DDQ	476nm	16-80	0.9979-0.9995	9
4	2,4-dinitrophenol , 3,5-dinitrosalycilic acid	446 nm, 435 nm	2.0-40	0.9992	10
5	Double distilled water-NaoH	219nm	2- 10	0.9980	11
6	Chloroform - Double distilled water	270 nm	2-12	0.9730	Proposed method

Table 5 : Comparisons of the proposed method with other existing methods for the assay of ceftriaxone in pharmaceutical formulations.

S.no	Reagents/Solvent	λMax	Beer's law limits µg mL^{-1}	Correlation coefficient (R^2)	Reference
1	Folin-Ciocalteu (FC) reagent	750 nm	2-36	0.9926	12
2	5% Para dimethyl amino benzaldehyde	490.6nm	5-25	0.998	13
3	NaOH- Variamine blue	556 nm	0.2-7.0	0 .9992	14
4	Ferric chloride	485 nm	5–120	0.9994	15
5	4-dimethylaminobenzaldehyde	397 nm	20-100	0.9996	16
6	Chloroform - Double distilled water	290 nm	4-16	0.9880	Proposed method

Note: Table 4-5, shown related to literature may be included in the introduction part as text by literature citation.

CONCLUSION

In this paper a simple, precise and more economical UV visible spectrophotometric method for the determination of flucloxacillin and ceftriaxone in bulk and pharmaceutical formulation has been developed and validated as per the International conference on Harmonization (ICH) guidelines.

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