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VALIDATED RP - HPLC METHOD FOR THE ESTIMATION OF FLUCLOXACILLIN SODIUM IN TABLET FORMULATIONS

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Flucloxacillin Sodium in Bulk and Pharmaceutical tablet Formulation. Isocratic elution at a flow rate of 1.0 ml/min was employed on symmetry Bondpeak C18 10 µm (3.9 x 300 mm) at ambient temperature. The mobile phase consisted of Acetonitrile: 0.0.1M Phosphate buffer in the ratio of 35:65/v/v. The UV detection wavelength was 273nm and 10µl sample was injected. The retention time for Flucloxacillin Sodium is 1.5min. The percentage RSD for precision and accuracy of the method was found to be 0.1%.The method was validated as per the ICH guidelines. The method was successfully applied for routine quality control analysis of pharmaceutical formulation.

Key Words: Flucloxacillin Sodium, RP-HPLC, UV detection, Recovery, Precise.

INTRODUCTION

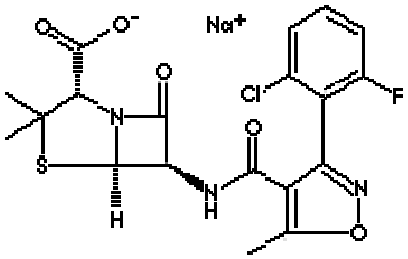

Fig: 1 Structure of Flucloxacillin Sodium

Flucloxacillin (INN) or floxacillin (USAN) is a narrow-spectrum beta-lactam antibiotic of the penicillin class. It is used to treat infections caused by susceptible Gram-positive bacteria. Unlike other penicillins, flucloxacillin

has activity against beta-lactamase-producing organisms such as Staphylococcus aureus[1] as it is beta-lactamase stable. it is ineffective against methicillin-resistant Staphylococcus aureus (MRSA).[2] Flucloxacillin has similar pharmacokinetics, antibacterial activity, and indications to dicloxacillin, and the two agents are considered interchangeable. It is reported to have higher, though rare, incidence of severe hepatic adverse effects than dicloxacillin,[3] but a lower incidence of renal adverse effects.[4] Flucloxacillin is indicated for the treatment of infections caused by susceptible bacteria. Specific approved indications include:[4][5] Flucloxacillin is contraindicated in those with a previous history of allergy to penicillins, cephalosporins, or carbapenems. It should also not be used in the eye, or administered to those with a history of cholestatic hepatitis associated with the use of dicloxacillin or flucloxacillin.[4] It should be used with caution in the elderly, patients with renal impairment where a reduced dose is required, and those with hepatic impairment, due to the risk of cholestatic hepatitis.[5] Dhiraj S. Nikam et.al.,[6] developed a RP-HPLC method for simultaneous determination of amoxicillin trihydrate and flucloxacillin sodium in bulk and pharmaceutical formulation. The separation was made by a Kromasil C18column (250 cm × 4.6 mm, 5µm) using 0.020 M potassium dihydrogen orthophosphate - acetonitrile (75:25) as mobile phase. The limits of quantification were approximately 0.16 µg/ml for amoxicillin trihydrate and 0.25 µg/ml for flucloxacillin sodium. Sarif Niroush Konari et.al.,[7]proposed a LC-analytical method. A chromatographic separation of the two drugs was achieved with a Thermosil C_{18} (4.6 mm × 250 mm, 5 µm) analytical column using potassium dihydrogen phosphate buffer (adjusted to pH 3 by ortho phosphoric acid): methanol (70:30%, v/v) in isocratic mode at a flow rate of 1 mL/min and column at ambient temperature. The detection was monitored at 225 nm using a PDA detector. The mean recovery value for fluc and amox was 99.9% and 99.7%, respectively. The limit of detection for fluc and amox was 0.018 and 0.009 µg/mL and the limit of quantification was 0.06 and 0.03 µg/mL, respectively. The retention time was observed at 2.582 and 3.407 min for amox and fluc, respectively. TuaniYT* et.al.,.[8] described Ampicillin and Cloxacillin in Oral suspension dosage forms. The chromatographic separation was attained on P. Hypersorb ODS (250 x 4.6) mm with precolumn as the stationary phase at ambient temperature. The mobile phase was composed of Acetonitrile and 0.02 M Phosphate buffer pH 3.0, 35:65 with a flow rate of 1.8 mL/min, and UV detection at 225 nm. The run time was 9 minutes. The injection volume was 20 µL. It can be used for the estimation of Ampicillin, Cloxacillin or Flucloxacillin in single as well as combination pharmaceutical dosage forms. A. V. D Nagendrakumar et.al.,[9] proposed a simple, selective, linear, precise, and accurate RP-HPLC method was developed and validated for rapid assay of Pizotifen in pharmaceutical dosage form. Isocratic elution at a flow rate of 1.0 mL/min was employed on Chromosil C18 (250mm × 4.6 mm, 5 μm) column at ambient temperature. The mobile phase consists of methanol: acetonitrile in the ratio of 10: 90 v/v. The UV detection wavelength was 230 nm, and 20 μL samples were injected. The retention time for Pizotifen was 2.019 min. The percent RSD for accuracy of the method was found to be 0.2603%. The method was validated as per the ICH guidelines.

MATERIAL AND METHODS

Instrumentation

Peak HPLC containing LC 20AT pump and variable wavelength programmable UV-Visible detector and Rheodyne injector was employed for investigation. The chromatographic analysis was performed on a Bondapak C18 5 µm (4.6 mm x 15 cm) Degassing of the mobile phase was done using a Loba ultrasonic bath sonicator. A Denwar analytical balance was used for weighing the materials.

Chemicals and solvents

The reference sample of Flucloxacillin Sodium was obtained from Cipla, Mumbai. The Formulation was procured from the local market. Phosphate buffer, KOH, Phosphoric acid and acetonitrile, HCL used were of HPLC grade and purchased from Merck Specialties Private Limited, Mumbai, India.

The mobile phase

A mixture of Acetonitrile: 0.0.1M Phosphate buffer in the ratio of 35:65/v/v was prepared and used as mobile phase.

Preparation of solutions

Preparation of 0.01 M Phosphate Buffer pH 7.0

Dissolve 2.72 g of Potassium Dihydrogen Orthophosphate in 2 L of water. Adjust to pH 7.0 with 45 % Potassium Hydroxide.

Standard Solution

Into a 100 ml volumetric flask, accurately weigh 150 mg Flucloxacillin Sodium Standard. Dissolve and dilute to volume with solvent. Dilute 10 ml of this solution to 50 ml with water.
Filter through 0.45 µm filter.

Sample solution:

Empty 20 capsules and determine the average mass. Into a 100 ml volumetric flask accurately weigh 160 mg of sample. Dissolve and dilute to volume with solvent. Dilute 10 ml of this solution to 50 ml with water. Filter through 0.45 µm filter. Prepare the mobile phase and set up the equipment as specified in the standard procedure. Inject the standard and sample preparations to test the system suitability to the following criteria:

Method Development

For developing the method, a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wave length and choosing stationary and mobile phases. The following studies were conducted for this purpose:

Detection of wavelength

The spectrum of 10 ppm solution of Flucloxacillin Sodium was recorded separately on UV spectrophotometer. The peak of maximum absorbance wavelength 273nm was observed.

Choice of stationary phase and mobile phase

Finally the expected separation and peak shapes were obtained on Bondpeak C18 10 µm (3.9 x 300 mm)

Flow rate

Flow rates of the mobile phase were changed from 0.5-1.5 ml/min for optimum separation. It was found from experiments that 1.0 ml/min flow rate was ideal for elution of analyte.

Validation Procedure and Requirements

The analytical performance of the method of analysis was checked for specificity, System suitability, detection limit, and method precision.

Specificity

The solutions were injected using the conditions specified in the method of analysis. Flucloxacillin is stable under UV exposure. No components are seen to co-elute with Flucloxacillin peak, and the peak purity results indicate that Flucloxacillin peak can therefore be considered spectrally pure. The method is specific for the assay of Flucloxacillin in the product. Chromatogram results were shown from Fig:2 to Fig:7 and peak purity results were shown Fig:8 to Fig:11.

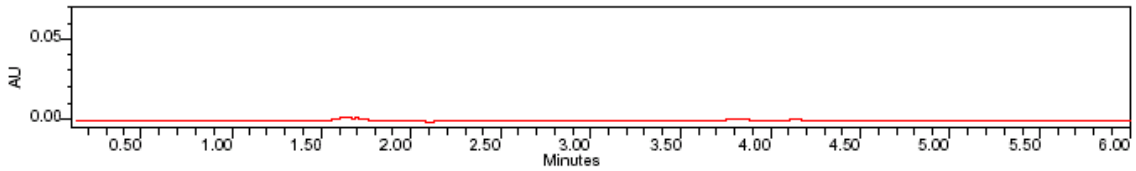

Fig: 2 Solvent – No significant peak detected

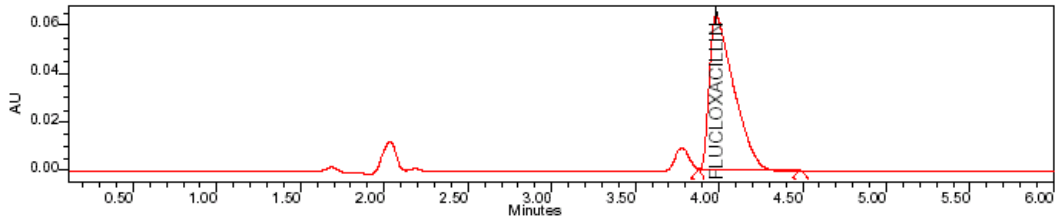

Fig: 3 Drug active – Peak due to Flucloxacillin eluted at 3.98 minutes

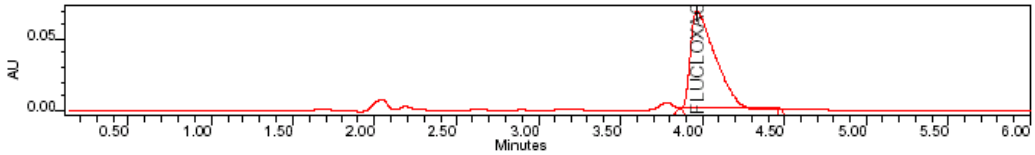

Fig: 4 Product – Peak due to Flucloxacillin eluted at 3.96 minutes.

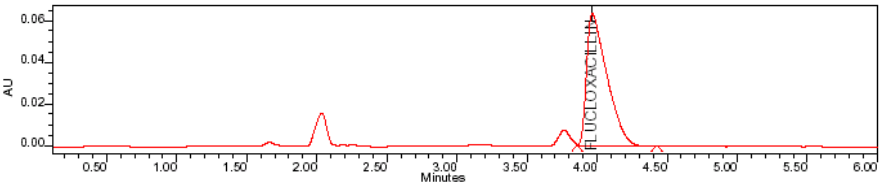

Fig: 5 Active – UV stress: Peak due to Flucloxacillin eluted at 3.97 minutes

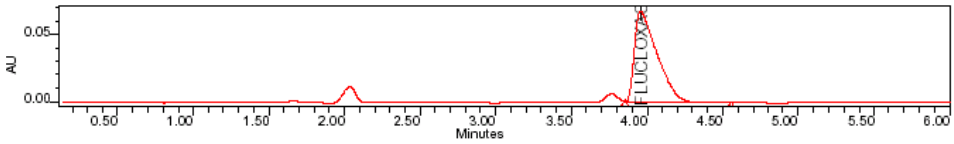

Fig: 6 Product – UV stress: Peak due to Flucloxacillin eluted at 3.96 minutes

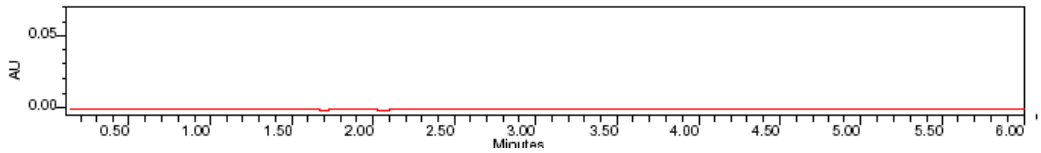

Fig: 7 Placebo – No significant peaks detected

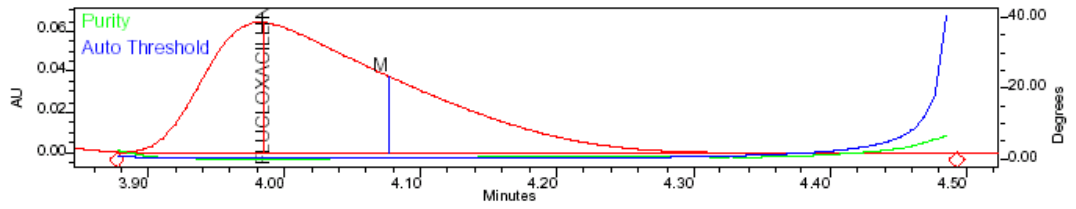

Fig: 8 Drug active: Purity angle < Threshold = 0.405 < 0.658

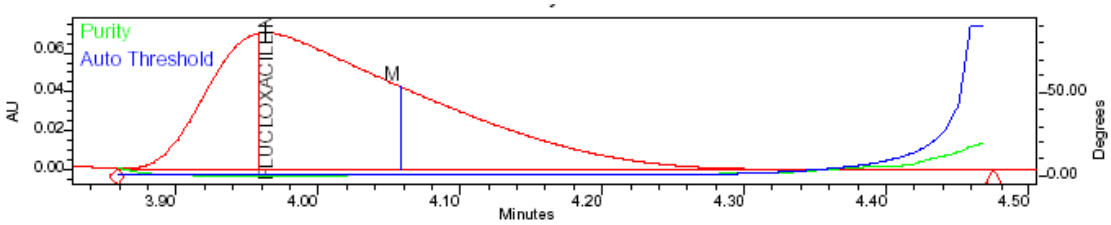

Fig: 9 Drug product Purity angle < Threshold = 0.787 < 0.828

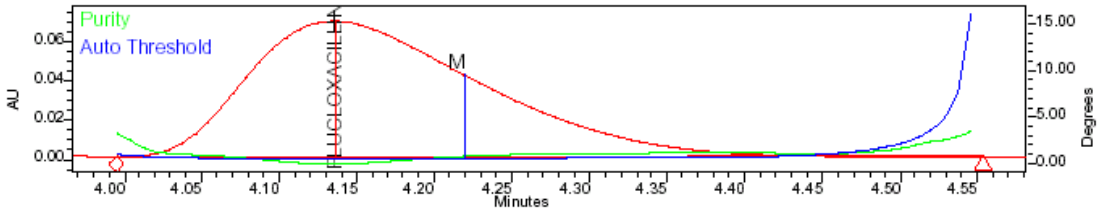

Fig: 10 Drug active Purity angle < Threshold= 0.560 < 0.658

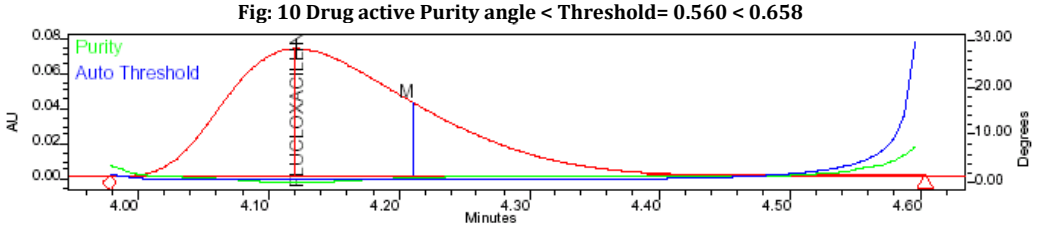

Fig: 11 Drug product Purity angle < Threshold = 0.681 < 0.753

System Suitability

System suitability is a measure of the performance and chromatographic quality of the total analytical system – i.e. instrument and procedure. The requirements for system suitability for this method are: The % RSD of the peak responses due to Flucloxacillin for the five replicate injections must be less than or equal to 2.0 %. The tailing factor of the peak due to Flucloxacillin must not be more than 2. The theoretical plate count must not be less than 2000. Five replicate injections of working standard solution were injected according to the method of analysis. The percentage relative standard deviation (% RSD) for the peak responses was determined. The analytical system complies with the requirements specified by the system suitability. Results are in the Table: 1.

Table:1 System Suitability Results

Sample	Flucloxacillin Area	Flucloxacillin Tailing	Flucloxacillin Tangent
1	766329	2.08	2748
2	766821	2.07	2743
3	765630	2.09	2754
4	765413	2.08	2778
5	767700	2.09	2773
Mean	766379	2.08	2759
% RSD	0.1		

Linearity

The linearity of an assay method is its ability to elicit test results, which are direct proportional to the concentrations of drug actives in samples in a given range. Proof of linearity justifies the use of single- point calibrations. The correlation coefficient of the regression line for Flucloxacillin should be greater than or equal to 0.999.The Y-intercept of the line should not be significantly different from zero, i.e. the assessment value (z) falls within the specified limits only when +2 > z > -2. Five solutions containing 50, 75, 100, 125, and 150 % of Flucloxacillin, relative to the working concentrations of 0.6032 mg/ml, were prepared and injected according to the method of analysis. A linear regression curve was constructed, and the correlation coefficients (R) and assessment values calculated. The correlation coefficient (R) for Flucloxacillin is 0.9999. The plot is a straight line, and the assessment value (z) falls within the specified limit at 1.84 for Flucloxacillin. The method is therefore linear within the specified range. Calibration Curve is shown in the Fig: 12 and Results are shown in the Table: 2.

CALIBRATION CURVE: y = Bx + A, R^2 = coeff. of determination

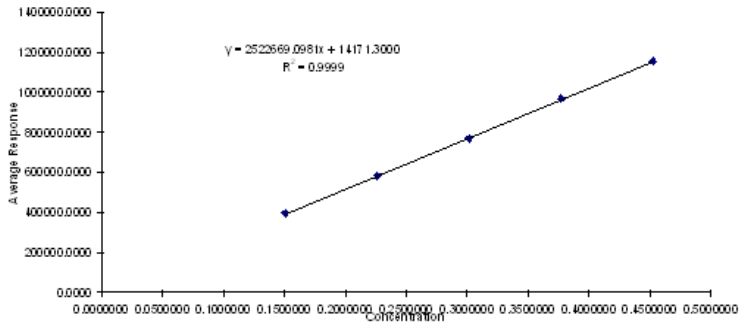

Fig: 12 Calibration Curve

Table: 2 Linearity results

Sample Number	Concentration	Response 1	Response 2	Average Response
1	0.1508000	397869.0000	394854.0000	396361.5000
2	0.2262000	584648.0000	584546.0000	584597.0000
3	0.3016000	770421.0000	771022.0000	770721.5000
4	0.3770000	969138.0000	965483.0000	967310.5000
5	0.4524000	1156004.0000	1156098.0000	1156051.0000

Accuracy

The accuracy of an analytical method expresses the closeness of test results obtained by that method to the true value. The percentage recovery of the active compounds, for each solution prepared, must be within 98 – 102 % of the actual amount. Sample solutions were spiked with known concentrations of Flucloxacillin to result in concentrations of 0.16 mg / ml, 0.24 mg / ml, 0.32 mg / ml, 0.4 mg / ml, and 0.48 mg / ml representing respectively 50, 75, 100, 125, 150 % of Flucloxacillin relative to the working concentration of 0.32 mg / ml. The above samples were injected in duplicate according to the method of analysis. From the accuracy results above, it passes the test. The percentage recovery values for Flucloxacillin satisfy the acceptance criteria for accuracy across the range of 50 % -150 %. The results are tabulated in Table:3.

Table: 3 Accuracy Results

Sample	Theoretical	Actual	% recovery	Average % recovery
50%	7.1500	7.0811	99.0	99
50%	7.1500	7.0421	98.5	
75%	10.3500	10.5259	101.74	102
75%	10.3500	10.5369	101.85	
100%	14.3000	14.0144	98.00	98
100%	14.3000	14.0772	98.44	
125%	17.5000	17.8135	101.81	102
125%	17.5000	17.7624	101.52	
150%	20.6921	20.8239	100.64	101
150%	206921	20.8670	100.85	

Table: 4 Repeatability Results

Sample number	Results (mg / cap)
	Flucloxacillin
1	261.2144
2	263.4527
3	261.8761
4	258.1499
5	262.7354
6	262.5501
Mean	261.7
% RSD	0.72

Method precision

The precision of an analytical procedure expresses the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of a homogenous sample.

Repeatability

This parameter determines the repeatability of assay results under the same operating conditions over a short period of time. The % RSD due to Flucloxacillin concentration for the six samples must be less than or equal to 2 %. Six separate sample preparations of batch 238917 were analysed according to the method of analysis. The % RSD due to Flucloxacillin concentration for the six samples meets the requirements for reproducibility at 0.72. The results are tabulated in Talbe:4.

Intermediate Precision

Intermediate Precision of an analytical procedure expresses intra-laboratory variations of the repeatability test performed by: by a different analyst, on a different day on a different instrument, and using different columns, reagents, mobile phases and solvents. The % RSD due to Flucloxacillin concentration for the six samples must be less than or equal to 2 %. The mean results obtained in the repeatability, and the intermediate precision must not differ by more than 3 %. Six separate sample preparations of batch 238917 were assayed according to the method of analysis. The % RSD for intermediate precision is 0.61 %. The intermediate precision and repeatability comply as they differ by 0.2 %. Results are tabulated in Table:5 and 6.

Table: 5 % RSD Results

Sample	Results (mg / cap)
	Flucloxacillin
1	258.9379
2	259.3967
3	260.9074
4	261.2728
5	261.6323
6	263.2872
Mean	260.9
% RSD	0.61

Table: 6 Repeatability and Intermediate Precision Results

Sample	Mean Results (mg / cap)
	Flucloxacillin
Repeatability	261.7
Intermediate Precision	260.9
Mean	261.3
% RSD	0.2

Range

Range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample (including this concentration) for which it has been demonstrated that the analytical procedure has a

suitable level of precision, accuracy and linearity. Based on the accuracy results, the range for the assay of Xixia – Flucloxacillin 250 mg is 125 – 375 mg / cap of Flucloxacillin, which represents 50 % to 150 % of the working concentration.

Declaration on the validity of the method

The method for the assay of Xixia – Flucloxacillin 250mg Capsules complies with the requirements for linearity, specificity, system suitability, method precision and accuracy across the range of 50 % to 150 %. The method is therefore acceptable as valid.

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