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Stability indicating RP-HPLC method for the estimation of flucloxacillin sodium in a tablet dosage form

Sonalika Patro*, S. Harshith kumar, M. Barath kumar, E.Masthaniah, K. Sairam, S. Prem Sumanth Kumar

Department of Pharmaceutical Analysis, Ratnam Institute of Pharmacy, Pidathapolur, Nellore, Andhra Pradesh, India.

ABSTRACT

A Simple, accurate and precise method was developed and validated for the determination of flucloxacillin sodium in its tablet dosage form. The separation was eluted on xterra c18 column (4.6x150mm, 5micron) using a mixture of octane buffer and methanol as mobile phase in a ratio of (30:70) which was pumped through column at a flow rate of 1ml/min. Optimised wavelength for flucloxacillin was 237nm, the retention time was 2.305minutes and the percentage purity was found to be 98.14%. System suitability parameters such as theoretical plate and tailing factor for flucloxacillin sodium was found to be 2991.64 and 1.90 respectively, the proposed method was validated as per ICH guidelines (ICH, Q2 AND (R1)) the method was found to be linear at the concentration range of 20-100µg/ml and the correlation coefficient (r2) value was found to be 0.9994 percentage RSD for precision was 0.9% and percentage RSD for ruggedness was 0.5%. The precision study was precise, robust and repeatable. The LOD and LOQ values are 2.98 and 9.98 respectively. Hence the suggested RP-HPLC method can be used for routine analysis for flucloxacillin sodium in tablet dosage form.

Keywords: RP-HPLC; Xterra C18 column; Flucloxacillin sodium; system suitability parameters; ICH guidelines; routine guidelines.

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Corresponding Author

Name: Sonalika Patro
Email: sonalikapatro07@gmail.com

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INTRODUCTION

Flucloxacillin sodium is chemically known as sodium;(2S,5R,6R)-6-((3-(2-Chloro-6-fluorophenyl)-5-methyl-1,2-oxazole-4-carbonyl)amino)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo (3.2.0)heptanes-2-carboxylate. It is an ßeta lactum antibiotic and it is used to treat skin infection, wound infections, chest infections and ear infections in children.

The literature review shows very few methods for flucloxacillin sodium estimation by Rp-Hplc method. Hence it felt that, there is need of development of new, precise and cost effective analytical method development for the estimation of flucloxacillin sodium in a tablet dosage form.

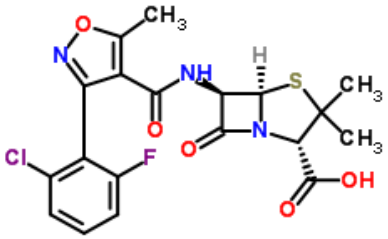

Figure 1: Structure of Flucloxacillin sodium

Present work is aimed to develop a new, simple, fast, rapid, accurate, cost effective, and reproducible method for the analysis of flucloxacillin sodium. The developed method was validated according to the ICH guidelines and degradation studies were performed.

MATERIALS AND METHODS

Chemicals used

Octane buffer, Water, Acetonitrile & methanol, Sodium hydroxide, Potassium di hydrogen ortho phosphate, Ortho phosphoric acid all chemicals where used HPLC grade only manufactured by Qualigens.

All the glassware's were used of borosilicate glass of class-A and all the solvents and prepared solutions were filtered through nylon filter 0.45µm.

Table 1: Instrument specification

S.NO	Instrument	Model/Company
1.	HPLC	WATERS Model:2965 with 2478 detector-Empower 2 software

2.	Ultra-soni-cator	SE60US/SCALETEC
3.	Electronic bal-ance	SAB224CL/ENERTECH
4.	Thermal oven	YAMTO
5.	pH meter	PH-7000/SMIS
6.	Filter paper 0.45 microns	MILLI PORE
7.	UV/VIS spec-trophotometer	LABINIDA UV 3000+
8.	Pipettes and burettes	Borosil

Details of marketed formulations

Label claim: Flucloxacillin sodium 500mg, Brand name: Staphonex-500mg tablet

Details of procured drugs

Flucloxacillin sodium was procured from Fortune pharma training institute, Hyderabad, India. It is used as standard drug it showed 98.14% of assay results.

Wave length selection

UV Spectrum of 10µg/ml for flucloxacillin sodium in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 237nm. At this wavelength flucloxacillin sodium shows good absorbance.

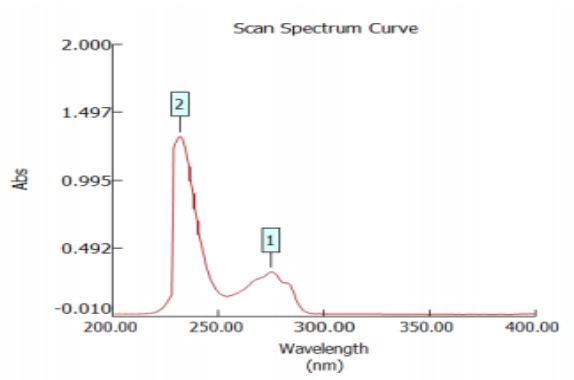

Figure 2: UV Spectrophotometry of flucloxacillin sodium

The chromatographic method development for the stimulation and estimation of flucloxacillin sodium were optimised in several trails for various parameters as different column, flow rate and mobile phase, finally the chromatographic method was selected for the separation and quantification of flucloxacillin sodium in tablet dosage form by RP-HPLC method.

Optimised chromatographic conditions

Temperature : Ambient (25ºc)
Modes of separation : Isocratic mode
Column : X terra C18 4.68x150mm, 5micron
Column temperature : Room temperature
Flow rate : 1.0ml/min
Injection volume : 20µl/min
Wavelength : 237nm
Run time : 10min
Mobile phase : Octane buffer: methanol (30:70)
Diluents : Mobile phase is used as diluents

Standard preparation

Accurately weigh and transfer 10mg of flucloxacillin sodium working standard into a 10ml clean dry volumetric flask add diluents and sonicate to dissolve it completely and make up the volume to the mark with the diluents. (Stock solution)

Further pipette out 0.6 ml of flucloxacillin sodium of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents. (60ppm)

Preparation of 0.1% octa sulphonic acid: (octane buffer)

To prepare 0.1% octa sulphonic acid, pipette out 1 ml octa sulphonic acid in 1000ml water. Adjust the pH up to 3.5 with sodium hydroxide solution.

Preparation of mobile phase

Mix a mixture of buffer 300ml (30%) and 700ml acetonitrile HPLC (70%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45µm filter under vacuum filtration.

Diluents preparation: Mobile phase is used as diluents.

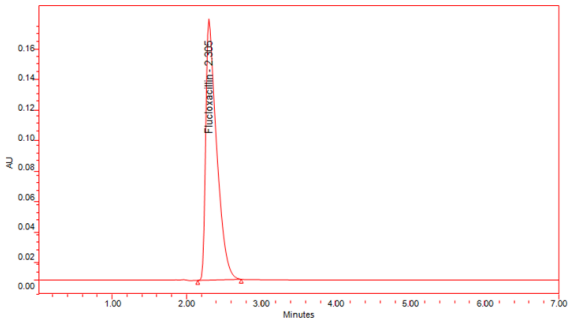

Figure 3: chromatogram of optimized flucloxacillin sodium

Assay

Standard and sample solutions are injected as described under experimental condition. The corresponding results are shown below.

$$\frac{AT}{AS} \times \frac{WS}{DT} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.Wt}{LC} \times 100$$

Where:

AT = Average area counts of test preparation, AS = Average area counts of standard preparation, WS = Weight of working standard taken in mg, P = Percentage purity of working standard, LC = Label claim of flucloxacillin sodium mg/ml (60mg)

RESULTS AND DISCUSSION

Method development

A reverse phase HPLC method was developed by considering the system suitability parameters i.e. resolution between the peaks, tailing factor (T), number of theoretical plates (N), run time and cost effectiveness. The optimised method developed resulted in the elution of flucloxacillin sodium at 2.305 and the total run time will be 10minutes with all the system suitability parameters.

System suitability results for flucloxacillin sodium was found at RT 2.305 with peak area of 1723973 Theoretical plates 2933.35 and Tailing/fronting factor 1.93.

The acceptance criteria for the tailing factor should be not more than 2.0 and for the theoretical plates should be not less than 2000

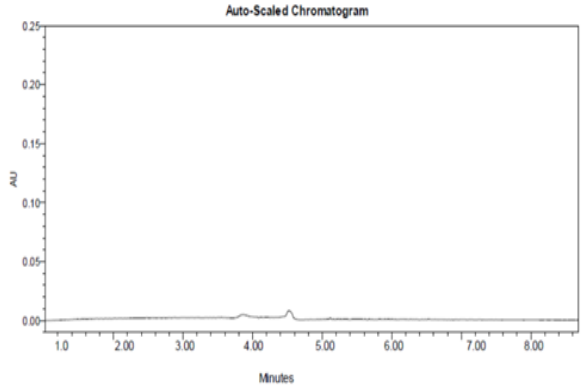

Figure 4: Chromatogram of blank

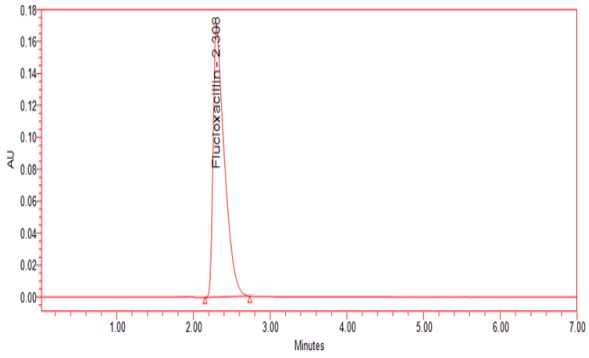

Figure 5: Chromatogram of standard

Method validation

Validation is a process that establishes by laboratory studies in which the performance characteristics of method meet the requirements for the intended analytical application. The RP-HPLC method developed was validated according to the ICH guidelines. The method was validated for the parameters in terms of system suitability, selectivity, linearity, accuracy, precision, ruggedness, robustness, and limit of detection (LOD), limit of quantification (LOQ).

Specificity

Specificity is carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study was performed by injecting blank and standard into the system. There is no inference of any peak in blank with the retention time of the analytical peaks.

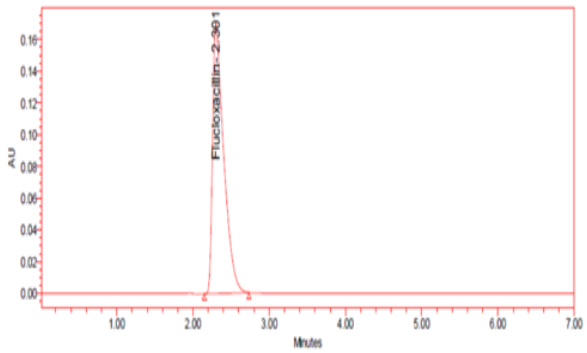

Figure 6: Chromatogram of sample

The specificity test was performed and there were no inference of impurities in the retention time of analytical peak.

Linearity

The linearity study was performed from the concentration of 20µg/ml to 100µg/ml. Each concentration was injected into chromatographic system. The area of each level was used for the calculation of correlation coefficient factor. The results are tabulated in table 2

Table 2: Linearity results for Flucloxacillin sodium

S.no	Linearity level	Concentration	Area
1.	I	20	76965
2.	II	40	152413
3.	III	60	218596
4.	IV	80	298643
5.	V	100	364351

Fig: 6 Linearity graph of Flucloxacillin sodium

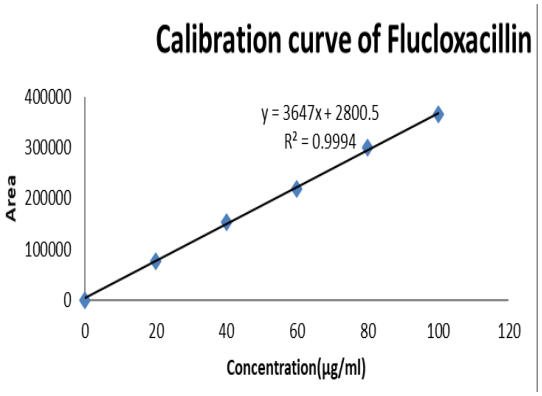

Figure 7: Linearity graph of Flucloxacillin sodium

The linearity study was performed from the concentration range of 20µg/ml to 100µg/ml and the correlation coefficient was found to be 0.9994.

Accuracy

The accuracy study was performed for 50%, 100% and 150% for flucloxacillin sodium and each level were injected into the chromatographic system. The

area of each level was used for the calculation of %recovery, the results are tabulated in table 3.

Table 3: Accuracy results for flucloxacillin sodium

% Concentration (At specification level)	Area	Amount Added (mg)	Amount Found (mg)	% recovery	Mean recovery
50%	801981.5	5	4.86	96.52	99.9
100%	913613.7	12.5	12.49	99.92	
150%	13478454.7	18.75	18.85	100.53	99.95
Injection-4	2.300	1729458			
Injection-5	2.300	1718752			
Average		1718805.3			
Standard deviation		8040.9			
%RSD		0.5			

The %RSD for the area of five injections should not be more than 2.0%.

Precision

Repeatability: The precision study was performed for five injections of flucloxacillin sodium. Each standard injection was injected into chromatographic system. The each area of standard injection is used for the calculation of %RSD. The results are tabulated in table 4.

Table 4: summarized precision results for flucloxacillin sodium

Injection	RT (Flucloxacillin sodium)	Area of flucloxacillin sodium
Injection-1	2.299	1693252
Injection-2	2.300	1718953
Injection-3	2.301	1721186
Injection-4	2.301	1725670
Injection-5	2.301	17133700
Average		1718552.4
Standard deviation		15226.1
%RSD		0.9

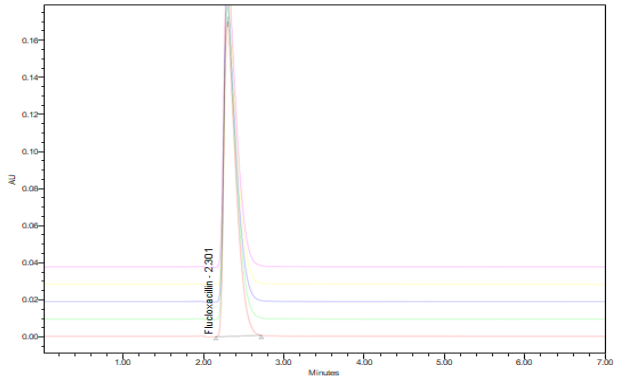

Figure 8: Precision overlay chromatogram

The %RSD for the area of five standard results should not be more than 2

Intermediate precision

The standard solution was injected for five times and the area is measured for all the five injections in HPLC.

Table 5: summarized ID precision results for flucloxacillin sodium

Injection	RT (Flucloxacillin sodium)	Area for flucloxacillin sodium
Injection-1	2.295	1707855
Injection-2	2.298	1722965
Injection-3	2.299	1715296

LOD (Detection of limit)

The detection of limit of flucloxacillin sodium was found to be 2.98, Acceptance criteria: S/N ratio value shall be 3 for LOD solution.

LOQ (Limit of quantification)

The quantification limit of flucloxacillin sodium is 9.98. Acceptance criteria: S/N ratio value shall be 10 for LOQ solution

Robustness

As a part of robustness, deliberate change in the flow rate, mobile phase composition, temperature, variation was made to evaluate the impact on the method.

The flow rate was varied at 0.9ml/min to 1.1ml/min: Standard solution 60ppm of flucloxacillin sodium prepared and analysed using the varied flow rates along with method flow rate.

On the evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly, Hence it indicates that the method robust even by change in flow rate ±10%. The method is robust only in less flow condition.

Table 6: System suitability results for flucloxacillin sodium

S.no	Flow rate (ml/min)	System suitability results		
		Retention time	USP plate count	USP Tailing
1	0.9ml/min	2.473	3140	1.9
2	1.0ml/min	2.305	2997	1.94
3	1.1ml/min	1.835	3115	1.8

The Organic composition in the mobile phase was varied ±10%

Standard solution 60µg/ml of flucloxacillin sodium was prepared and analysed using the varied mobile phase composition along with actual mobile phase composition in the method.

The results are summarized

On the evaluation of above results, it can be concluded that the variation in 10% organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in mobile phase ±10%.

Table 7: change in organic phase results for flucloxacillin sodium

S.no	System suitability parameters

	Change in the Organic composition In mobile phase	Retention time	USP Plate count	USP Tailing
1.	10% less	2.398	3321	1.9
2.	*Actual	2.305	2997	1.94
3.	10% more	1.828	3315	1.8

Forced degradation studies

Accurately weigh and transfer 10mg of flucloxacillin sodium working standard into a 10ml clean dry volumetric flask add diluents and sonicate to dissolve it completely and make up the volume up to the mark with stock solution.

Hydrolytic degradation under acidic condition

Pipette 0.6ml of flucloxacillin sodium of the above stock solution into a 10ml volumetric flask added about 3 ml of 0.1N Hcl and sonicated for 10minutes and kept it in darkness for 12 hours then refluxed under heat at 60ºc in heating mantle for 1 hour. Neutralize the sample solution using 0.1N NaoH and diluted up to the mark with diluents. The final sample was filtered through 0.44 micron injection filters and injected into HPLC system.

Hydrolytic degradation under alkaline condition

Pipette 0.6ml of flucloxacillin sodium of the above stock solution into a 10ml volumetric flask add about 3ml of 0.1N NaoH and sonicated for 10minutes and kept it in darkness for 12hours then refluxed under heat at 60ºc in a heating mantle for 1 hour. Neutralize the sample solution using 0.1N Hcl and diluted up to the mark with diluents. The final sample was filtered through 0.44 micron injection filters and injected into HPLC system.

Thermal degradation

Pipette 0.6ml of flucloxacillin sodium of the above stock solution into a 10ml volumetric flask and kept in oven under heat at 105ºc for 12 hours and diluted up to the mark with diluents. The final sample was filtered through 0.44 micron injection filters and injected into HPLC system

Peroxide degradation

Pipette 0.6ml of flucloxacillin sodium of the above stock solution into a 10ml volumetric flask added 3ml of 3% hydrogen peroxide (H2O2) and sonicated for 10minutes and kept in darkness for 12hours and refluxed under heat at 60ºc in a heating mantle for 1 hour. The final sample was filtered through 0.44 micron injection filters and injected into HPLC system.

Photo degradation

Pipette 0.6ml above stock solution into a 10ml volumetric flask and expose to sunlight for 24hours and the volume was made up to mark with the diluents. Filter the solution with 0.45 microns syringe filters and place in vials.

Table 8: Degradation results of flucloxacillin sodium

	Flucloxacillin sodium	
	Area	%Degraded
Standard	1720758	
Acid	1626465	5.479736
Base	1649322	4.151426
Peroxide	1651577	4.020379
Thermal	1668536	3.034825
Photo	1551478	9.837525

Table 9: Summarized results of method validation

Parameters	Flucloxacillin sodium	Limit
Linearity range (µg/ml)	0-1000µg/ml	R^2<1
Regression coefficient (R^2)	0.999	R^2<1
Regression equation (Y=mx+c)	Y=3647x + 2800.5 R^2=0.9994	R^2<1
Assay	98.12%	90-100%
Specificity	Specific	No interference
Precision	0.9	NMT 2.0%
Intermediate precision (%RSD)	0.5	NMT 2.0%
Accuracy (% recovery)	99.95%	98-102%
LOD	2.98	NMT 3
LOQ	9.98	NMT 10

CONCLUSION

From the above experimental results and parameters it was concluded that, A new method as established for flucloxacillin sodium by RP-HPLC method. The chromatographic conditions were successfully developed and results are given below for the separation of flucloxacillin sodium by using Xterra C18 4.6x150mm, 5micron, flow rate was 1.0ml/min, mobile phase was octane buffer: methanol (30:70)

The proposed method was found to be simple, specific, precise, accurate, rapid and economical for the determination of flucloxacillin sodium in pharmaceutical dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness. The results will be validated statistically according to the ICH guidelines. The simple recoveries in all the formulations were in good agreement with their respective label claims. Hence the suggested RP-HPLC method can be used for routine analysis of flucloxacillin sodium in API and pharmaceutical dosage form.

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