Development and Validation of an Ultraviolet–Visible Spectroscopy Method in Vitro Determination of Flucloxacillin Capsule

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Abstract— The objective of the present study was to develop and validate a discriminative dissolution method for evaluation of Flucloxacillin Sodium as Flucloxacillin. Different conditions such as type of dissolution medium, volume of dissolution medium and rotation speed of paddle were evaluated. The best in vitro dissolution profile was obtained using Apparatus II (paddle), 75 rpm, 900 ml of pH 6.8 phosphate buffer as dissolution medium. The drug release was evaluated by UV-Visible Spectrophotometer method. The dissolution method was validated according to current ICH and FDA guidelines using parameters such as the specificity, accuracy, precision and stability were evaluated and obtained results were within the acceptable range. A simple, selective, rapid, and precise UV-Visible Spectrophotometer method has been developed for the in vitro determination of Flucloxacillin from dosage forms.

Keywords: UV-Visible Spectrophotometer; Flucloxacillin; Flucloxacillin Sodium; In vitro

I. INTRODUCTION

The Analytical method for Dissolution Flucloxacillin[1] Capsules 250 mg by UV-Visible Spectrophotometer is a non-compendial method. Therefore, the method of analysis for Dissolution of Flucloxacillin was validated[4-7] as per approved Protocol.

II. OBJECTIVE

To establish the documented evidence that the UV-Visible Spectrophotometer method for Dissolution of Flucloxacillin in Flucloxacillin Capsules 250 mg suitable for intended purpose. This validation Report covers the procedure for validation of the UV-Visible Spectrophotometer method for Dissolution of Flucloxacillin Capsules 250mg The validation was carried out in compliance with the ICH-Guidelines[2-3].

III. RESOURCES USED FOR VALIDATION

A. *Instruments:*

SN	Equipment Used	Equipment ID
	Analytical Balance	QC-BAL01, QC-BAL04
	UV-Visible Spectrophotometer	QC-UVS01
	pH Meter	QC-PCM01
	Dissolution Apparatus	QC-DIS01

B. *Chemicals / Reagents Used:*

Potassium Di Hydrogen Ortho Phosphate-AR Grade
Water-UV-VISIBLE SPECTROPHOTOMETER Grade
Sodium Hydroxide-AR Grade
Purified Water

C. *Standards, Sample and Placebo:*

Material Name	Batch No.	% Potency
Flucloxacillin Sodium WS	WS.04.00.18	95.4
Flucloxacillin Sodium WS	WS.02.00.19	94.9
Flucloxa 250mg	190044	NA
Placebo	FLUCLOXA250/Placebo	NA

IV. ANALYTICAL METHOD DETAILS

A. *Reagents:*

Potassium Di Hydrogen Ortho Phosphate-AR Grade
Water-UV-Visible Spectrophotometer Grade
Sodium Hydroxide-AR Grade
Purified Water

B. *Dissolution Parameters:*

Apparatus : USP, Type II Paddle with sinkers
Medium : 900mL Phosphate Buffer pH 6.8
Temperature : 37 ± 0.5 C
Speed : 100 rpm
Time : 45 minutes

C. *Preparation of Dissolution Medium*

1) Phosphate Buffer pH 6.8:
Dissolve 6.8gm of Potassium dihydrogen orthophosphate in 1000 ml of water and adjust the pH 6.8±0.02 with dilute sodium hydroxide solution.

D. *Preparation of standard solution:*

Weigh and transfer accurately about 60mg of Flucloxacillin Sodium working standard in to 200ml volumetric flask. Add 150 ml of dissolution medium, sonicate to dissolve and make up the volume to 200ml with dissolution medium and mix.

E. *Preparation of sample solution:*

Drop one capsule in each six dissolution vessels and run the dissolution apparatus. At specified time interval withdraw 10 mL sample from each dissolution vessel. Filter through 0.45 µm membrane filter by saturating the filter slowly and discard first 2-3mL filtrate.

F. *Procedure:*

Measure the absorbance of blank in single, Standard in triplicate and sample in single by using Standard 10mm Quartz Cuvette as per following sequence:

1) Diluent Blank (Blank 1):
Transfer dissolution medium into the cuvette and auto zero the absorbance of this solution. After auto zero record the absorbance of solution.

2) Empty Capsule Shell Solution (Blank 2):
Transfer the solution into the cuvette and record the absorbance of solution.
3) Standard Solution:
Transfer the Standard Solution into the cuvette and record the absorbance of solution.
4) Sample Solution:
Transfer the Sample Solution into the cuvette and record the absorbance of solution.

G. Determination of Content:

Calculate the % content of Amoxicillin dissolved by using following Formula. Each mg of Flucloxacillin Sodium is equivalent to 0.9538 mg of Flucloxacillin.
Calculation for Flucloxacillin:
% Drug Dissolved =

$$\frac{T(a) - \text{Blank 2 (a)} \times Sd \times \%\ \text{Potency of WS} \times 0.9538}{Sr \times Td \times \text{Label Claim}}$$

Where,
T(a) : Absorbance of test solution.
Blank 2 (a) : Absorbance of empty capsule shell solution.
Sd : Standard dilution
Sr : Standard response
Td : Test dilution

H. Precautions:

Rinse the cuvette twice with the solution to be measured before measuring the absorbance.

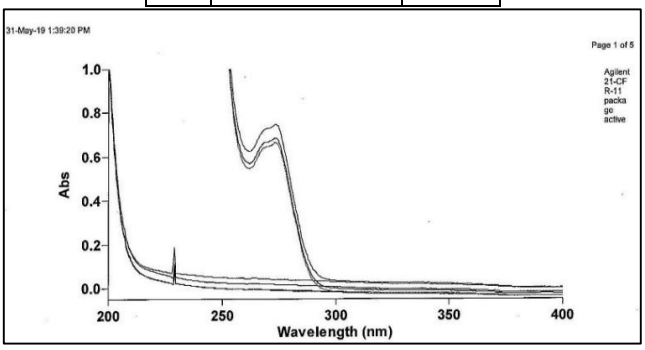
After completion of the analysis wash the cuvette with purified water and finally rinse with methanol. Air dry the cuvette before storage.

V. Validation Procedure for Analytical Performance Characteristics

A. Specificity:

1) Selectivity:
Blank, Standard and Sample solution were analyzed as per Methodology.

SN	Analyte Name	λ max
	Flucloxacillin	274



2) Acceptance Criteria:
λ max of Flucloxacillin in standard and sample should be comparable.
3) Conclusion:
λ max of Flucloxacillin in standard and sample found comparable. Therefore, the UV-visible spectrophotometer method for Dissolution determination of Flucloxacillin Capsules 250 mg is selective.

4) Placebo Interference:
Placebo was prepared and analyzed as per method and checked the interference with blank and sample.
5) Acceptance Criteria:
Placebo should not exhibit any peak at λ max of main peaks.
6) Conclusion:
Placebo does not exhibit any peak at λ max of main peaks.
7) Blank/Diluent Interference:
Reviewed the chromatograms in selectivity experiment.
8) Acceptance Criteria:
Blank/Diluent should not show any interference at λ max of main peaks.
9) Conclusion:
Blank/Diluent show no interference at λ max of main peak. Therefore, Dissolution method is selective.

B. Precision:

1) System Precision:
a) Experiment:
Five replicate measurement of the standard solution were examined in the UV-Visible Spectrophotometer.
b) System Precision Observations:

Absorbance	Absorbance of
	0.7257
	0.7243
	0.7246
	0.7257
	0.7251
Mean	0.7250
SD	0.00
RSD	0.09

c) Acceptance Criteria:
The Standard Deviation and % Relative Standard Deviation of the five replicate injections shall be calculated and reported.
d) Conclusion:
% RSD for five replicate injections of the standard solution was observed 0.09 % Flucloxacillin. Therefore, the UV-Visible Spectrophotometer method for Dissolution determination of Flucloxacillin Capsules 250 mg is precise.
2) Method Precision:
a) Experiment:
Prepared and analyzed six samples as per methodology.
b) Method Precision Observations:

Sample ID	Dissolution % of Claim Flucloxacillin
	102
	103
	103
	98
	103
	104
Mean	102.17
SD	2.14
RSD	2.09

c) Acceptance Criteria:
% RSD for six Dissolution values should not be more than 5.0%.

d) Conclusion:
% RSD for six Dissolution values was observed 2.09 % of Flucloxacillin. Therefore, the UV-Visible Spectrophotometer method for Dissolution determination of Flucloxacillin Capsules 250 mg is Precise.

3) Ruggedness (Intermediate Precision):

a) Experiment:
Prepared six samples of Flucloxacillin Capsules 250 mg were prepared and injected into the UV-Visible Spectrophotometer as per the method described under methodology. This study was done by different analyst on different day with respect to method precision.

b) Variables taken for Ruggedness:

SN	Variable	Analyst-1	Analyst-2
	Name	Aviral	May Thu

c) Ruggedness Results: Flucloxacillin

Sample ID	Analyst-1	Analyst-2
	102	98
	103	103
	103	100
	98	103
	103	100
	104	99
Mean	102.17	100.50
SD	2.14	2.07
%RSD	2.09	2.06
Overall Mean		101.33
Overall SD		2.19
Overall %RSD		2.16

d) Acceptance Criteria:
% RSD for six Dissolution values should not be more than 5.0% and overall %RSD for twelve Dissolution values under method precision and Intermediate Precision should not be more than 5.0%.

e) Conclusion:
% RSD for six Dissolution values was observed 2.06 % Flucloxacillin and for twelve vales was observed 2.16% Flucloxacillin. Therefore, the UV-Visible Spectrophotometer method for Dissolution determination of Flucloxacillin Capsules 250 mg is Rugged.

C. Linearity and Range:

1) Experiment:
Injected the solutions of concentrations in the range 20% to 150% of standard concentration.

2) Linearity Results: Flucloxacillin

SN	Concentration in%	Concentration in mcg/mL	Absorbance Response
	20	54.62	0.1381
	50	136.54	0.3504
	80	218.47	0.5644
	100	273.09	0.7074
	120	327.70	0.8545
	150	409.63	1.058
Correlation Coefficient			0.99996

3) Acceptance Criteria:
Correlation coefficient should not be less than 0.99.

4) Conclusion:
Correlation Coefficient 0.99996. Spectrophotometer method for Dissolution determination of Flucloxacillin Capsules 250 mg is Linear

D. Accuracy (Recovery):

Experiment: Placebo of Flucloxacillin Capsules 250 mg was spiked with Flucloxacillin at 50%, 100% and 150% in triplicate.

1) Accuracy Results: Flucloxacillin

a) Acceptance Criteria:
Mean recovery at each level should be in the range of 95.0% to 105.0% and Overall % RSD should not be more than 2.0%.

b) Conclusion:
Mean recovery for Flucloxacillin 97.9 % were observed and overall % RSD was observed 0.78% respectively. Therefore, the UV-Visible Spectrophotometer method for Dissolution determination of Flucloxacillin Capsules 250 mg is Accurate.

Sample ID	Amount Added (mcg)	Amount Recover (mcg)	% Recover	Mean Recover
50% SET-1	139.198	137.945	99.1	98.3
50% SET-2	139.087	137.244	98.7	
50% SET-3	138.895	136.395	98.2	
100% SET-1	277.911	271.241	97.6	97.7
100% SET-2	277.224	271.758	98.0	
100% SET-3	277.386	270.097	97.4	
150% SET-1	417.292	406.640	97.4	97.2
150% SET-2	416.978	404.463	97.0	
150% SET-3	416.827	405.496	97.3	
Mean				97.9
SD				0.76
%RSD				0.78

E. Robustness:

Experiment: Three sample solutions were prepared and analyzed as per proposed methodology with following variations in chromatographic parameters.
Change in λ max (± 1.0 nm): Flucloxacillin

Robustness under Change in λ max (± 1.0 nm)			
SN	Sample ID	Dissolution (%w/w) (-1.0 nm)	Dissolution (%w/w) (+1.0 nm)
	Control Sample-1	102	102
	Control Sample-2	103	103
	Control Sample-3	103	103

	Robustness Sample-1	98	98
	Robustness Sample-2	98	98
	Robustness Sample-3	99	99
	Mean	100.5	100.5
	SD	2.43	2.43
	%RSD	2.42	2.42

Change in pH of Dissolution media by ± 0.2: Flucloxacillin

Robustness under Change in pH of Buffer by ± 0.2			
SN	Sample ID	Dissolution (%w/w) (-0.2pH)	Dissolution (%w/w) (+0.2pH)
	Control Sample-1	102	102
	Control Sample-2	103	103
	Control Sample-3	103	103
	Robustness Sample-1	98	97
	Robustness Sample-2	101	98
	Robustness Sample-3	99	97
	Mean	101.0	100.0
	SD	2.10	2.97
	%RSD	2.05	2.97

Change in Dissolution Medium Temperature by ±1°C:

Robustness under Change in Column Oven Temperature by ± 5°C			
SN	Sample ID	Dissolution (%w/w) (-1°C)	Dissolution (%w/w) (+1°C)
	Control Sample-1	102	102
	Control Sample-2	103	103
	Control Sample-3	103	103
	Robustness Sample-1	98	96
	Robustness Sample-2	98	98
	Robustness Sample-3	96	96
	Mean	100.4	99.67
	SD	3.03	3.39
	%RSD	3.03	3.40

Changes in Dissolution RPM, (±4%):

Robustness under Change in Organic Phase Composition of Mobile Phase, (±2% Absolute):			
SN	Sample ID	Dissolution (%w/w) (-2%) Composition	Dissolution (%w/w) (+2%) Composition
	Control Sample-1	102	102
	Control Sample-2	103	103
	Control Sample-3	103	103
	Robustness Sample-1	104	103
	Robustness Sample-2	102	103
	Robustness Sample-3	100	104
	Mean	102.3	103.0
	SD	1.37	0.63
	%RSD	1.34	0.61

1) Acceptance Criteria:

System suitability criteria should be met under all conditions. No interference should be seen with blank/placebo at retention time of analyte. Overall RSD of Dissolution values obtained under control condition (Method precision) and Dissolution values obtained under each robustness condition should not be more than 5.0%.

2) Conclusion:

No interference was observed with blank/placebo at retention time of analyte. Dissolution values obtained under control condition and Dissolution values obtained under each robustness condition should were found within 5.0%.

F. Stability of Analytical Solution:

1) Experiment:

Prepared standard and sample in single as per the methodology and stored at room temperature. These preparations were analyzed initially and after specified time intervals as per the methodology.

Observations for Stability of Analytical Solution at Room Temperature: Flucloxacillin

Time	Abs. of Std	% Correl. for Std	% Dissolution Value	% Correl for Sample
Initial	0.7448	---	98	---
24 hrs	0.7374	99.01	99	101.02
48 hrs	0.7270	97.61	98	100.00

2) Acceptance Criteria:

Standard Solution: Correlation of Dissolution of old standard against freshly prepared Standard should be between 98.0%-102.0%.

3) Sample Solution:

Correlation of Dissolution of old sample solution against initial Dissolution should be between 98.0-102%.

4) Conclusion:

Standard solution was monitored up to 24 hrs and sample solution was monitored up to 48 hrs and found stable.

G. Filter Equivalency:

Experiment: Samples of Flucloxacillin Capsules 250 mg were prepared as per proposed methodology for filter Equivalency. Solutions were Centrifuged and filtered through different types of filters and then analyze as per the methodology.

Centrifuged sample

0.45 µm nylon filter.

0.45 µm PTFE filter.

Observations for the Filter Equivalency: Flucloxacillin

Filter Equivalency			
SN	Centrifuged	0.45 µm nylon filter	0.45 µm PTFE filter.
	101	99	100
	101	99	99
	101	100	99
Mean	101	99.33	99.33
RSD	0.00	0.58	0.58
Correlation	---	98.35	98.35

1) Acceptance Criteria:

Correlation of mean Dissolution value of the filtered samples and mean Dissolution value for Centrifuge sample should be between 98.0-102.0%.

2) Conclusion:

Filters were found suitable and correlation of mean Dissolution value of the filtered samples and mean Dissolution value for Centrifuge sample found between 98.0-102.0%.

H. System Suitability:

System suitability was evaluated by measuring the absorbance of standard solution throughout the validation.

Absorbance ID	Absorbance of Flucloxacillin
	0.7257
	0.7243
	0.7246
Mean	0.7249
SD	0.00
RSD	0.10

1) Acceptance Criteria:

System suitability criteria should be met under all conditions. Relative standard deviation for three replicates absorbance of standard solution should not be more than 2.0 %.

2) Conclusion:

The System suitability data revealed that system is suitable under all the performance characteristics outlined in the method.

VI. SUMMARY OF ANALYTICAL METHOD VALIDATION

Validation Parameters	Acceptance Criteria	Conclusion
Specificity		
Selectivity	λ max of Flucloxacillin in standard and sample should be comparable.	λ max of Flucloxacillin peak in standard and sample found comparable.
Blank /Diluent and Placebo Interference	Mobile phase/diluent and Placebo should not show any peak at the λ max of Flucloxacillin.	Blank/Diluent should not show any interference at λ max of main peak. Placebo does not exhibit any peak at λ max of main peak.
Precision:		
System Precision	%RSD for standard solution of Flucloxacillin absorbance should not be more than 2.0%.	% RSD for Flucloxacillin, 0.09
Method Precision	%RSD for the six results Dissolution of Flucloxacillin in Flucloxa250 Capsule should not be more than 5.0 %.	% RSD for Flucloxacillin, 2.09
Ruggedness	RSD of Dissolution values obtained for the six samples should not be more than 5.0%. Overall RSD of Dissolution values obtained under Method Precision and Intermediate Precision should not be more than 5.0%.	% RSD for Flucloxacillin, 2.16
Linearity and Range	Correlation coefficient should not be less than 0.99.	Correlation Coefficient 0.99996
Accuracy / Recovery	Mean recovery at each level should be in the range of 98.0% to 102.0% and Over all% RSD for 9 determinations should not be more than 2.0%.	Mean recoveries are in the range of 98.0% to 102.0% and Over all% RSD values are and 0.78%
Robustness:	System suitability criteria should be met under all conditions. Overall RSD of Dissolution values obtained under control condition (Method precision) and Dissolution values obtained under each robustness condition should not be more than 5.0%.	Complies
Stability in Analytical Solution:	Standard Solution: Correlation of Dissolution of old standard against freshly prepared Standard should be between 98.0%-102.0%.	Standard found stable up to 24 hrs. at 25°C and Sample solutions found stable up to 48 hrs. at Room temperature i.e. 25°C

	Sample Solution: Correlation of Dissolution of old sample solution against initial Dissolution should be between 98.0-102%.	
System Suitability	% RSD for three replicates absorbance of Standard solution is NMT 2.0%.	Complies
Filter Equivalency	Correlation of mean Dissolution value of the filtered samples and mean Dissolution value for Centrifuge sample should be between 98.0-102.0%.	0.45 µm nylon and 0.45 µm Teflon filters found suitable.

VII. ABBREVIATIONS

- ICH : International Council on Harmonization
- BPC : British Pharmacopoeia Certified Reference Standard
- Std : Standard
- RSD : Relative Standard Deviation
- Correl. : Correlation
- NMT : Not More Than
- NLT : Not Less Than
- WS : Working Standard
- SN : Serial Number
- API : Active Pharmaceutical Ingredient

VIII. CONCLUSION

The Method was validated for Specificity, Linearity, Range, Precision, Accuracy, Ruggedness, Robustness, Stability of Analytical solutions and Filter Equivalency. All the parameters were found to meet the specified acceptance criteria as per approved protocol. Based upon the Validation data it is concluded that the method for Dissolution determination of Flucloxacillin Capsules 250 mg is Specific, Linear, Precise, Accurate, Rugged, Robust and Stable. Therefore, this method is suitable for routine analysis of the Dissolution of Flucloxacillin Capsules 250mg.

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