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Analytical Method Validation of Norfloxacin Tablet 400 mg Dosage form for Dissolution Test

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ABSTRACT

Validation of an analytical method is the process that establishes, by laboratory studies, that the performance characteristic of the method meets the requirements for the intended analytical applications. A document describing the analytical standard test method validation for the dissolution of Norfloxacin in Norfloxacin Tablet 400mg, the validation parameters and the acceptance criteria pertinent to the validation study. Process of method validation consists of the parameter stages such as Specificity, Accuracy, Linearity and Range, Precision or Reproducibility and Ruggedness.

Key words: Norfloxacin, UV, Validation, Dissolution.

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INTRODUCTION

Norfloxacin is chemically 3-Quinolinecarboxylic acid, 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid.

This document presents a discussion of the characteristics for consideration during the validation of the analytical procedures for the Norfloxacin Tablet 400 mg. The discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures such as Identification tests; Quantitative tests of the active moiety in samples of drug substance or drug product; Limit tests for the control of impurities; Quantitative tests although there are many other analytical procedures, such as dissolution testing for drug products [1].

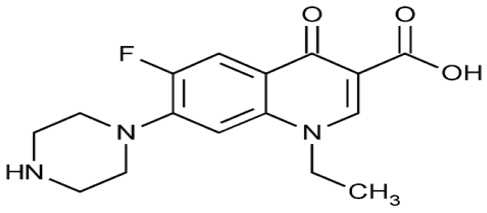
The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose [1].

MATERIALS AND METHODS

Chemicals & Reagents:

Norfloxacin API was received from M/s C.T. Shah & Company as a gift sample for the study. Norfloxacin tablets, claimed to contain 400mg of Norfloxacin received from M/s C.T. Shah & Company.

Stricture of Norfloxacin:



$C_{16}H_{18}FN_3O_3$

Instruments:

pH Meter from Lab India, UV- Visible spectrophotometer make Shimadzu, Dissolution Test apparatus make Lab India.

METHODOLOGY:

Dissolution of Norfloxacin:

Conditions of testing

Apparatus : Paddle (USP apparatus [2])

Speed	: 50 rpm
Medium	: 750 ml Buffer with pH 4.0
Temperature	: 37°C ± 0.5°C
Sample withdrawal time	: 30 min

Preparation of buffer solution:

To 1000 ml of water add 2.86 ml of glacial acetic acid and 1 ml of 50% (w/w) sodium hydroxide solution. If required adjust the pH to 4.0 with the help of glacial acetic acid or sodium hydroxide solution.

Preparation of Standard Solution:

Weigh accurately about 50 mg of Norfloxacin working standard in 100 ml volumetric flask, dissolve in dissolution medium and dilute to volume with dissolution medium. Dilute 1 ml of obtained solution in 100 ml volumetric flask with dissolution medium.
(Concentration of Norfloxacin is 0.005 mg/ml or 5 ppm).

Method and Calculation:

Carry out the determination for 6 individual tablets.

Place the 750ml of Buffer with pH 4.0, into each of the vessel of the apparatus; equilibrate the dissolution medium to 37°C ± 0.5C°. Place 1 tablet in each of the vessel taking care to exclude the air bubbles from the surface of tablets and immediately operate the apparatus at 50 RPM.

Withdraw sample after 30 minutes from each vessel from a zone midway between the surface of the dissolution medium and the top of the rotating blade, not less than 1 cm away from the vessel wall.

Filter the sample through membrane filter having pore size not more than 0.45 mcm discarding the first portion of filtrate.

Dilute 1ml of filtrate in 100 ml volumetric flask with dissolution medium and mix (test preparation).

Measure the absorbance of the standard preparation and sample preparations on spectrophotometer at 278 nm using dissolution medium as blank.

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Calculate the quantity of Norfloxacin released in % as follows

Calculation:

$$mg/tab = \frac{Spl\ abs}{Std\ abs} \times \frac{Wstd.}{100} \times \frac{1}{100} \times \frac{750}{1} \times \frac{100}{1} \times \frac{P}{100}$$

$$\% \text{ of label claim} = \frac{Mg/Tab}{Label\ claim} \times 100$$

Where,

Spl abs: Absorbance of sample;
Std abs: Absorbance of standard;
Wstd : Weight of Norfloxacin WS in mg;
P : As such potency of standard.
Limit: Not less than 80% (Q) in 30 minutes

METHOD VALIDATION [3]**:**

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

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Precision or Reproducibility

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

Ruggedness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

RESULTS AND DISCUSSION

Specificity

This study is to be carried out to determine the interference due to placebo. Placebo preparation will analyzed using the method of analysis.

Specificity for Norfloxacin Tablets 400mg:

Table – 1

Standard Addition and Recovery				
Sr. No.	**Test Sample**	**Weight Taken**	**Absorbance**	**Remarks**
1	Placebo 1	212.1	0.002	No Interference
2	Placebo 2	212.3	0.001	
3	Placebo 3	212.2	0.001	
4	Placebo 4	212.2	0.003	
5	Placebo 5	211.8	0.002	
6	Placebo 6	212.4	0.001	

Accuracy:

Standard Addition and Recovery
Working standard Potency : 99.44%
Working standard ID : 09/WS-04
Standard Addition and Recovery: Norfloxacin Tablet 400mg

Table-2

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Standard Addition and Recovery Results					
Concentration	**Weight of**	**Added**	**Recovery of**	**%Recovery**	**Mean and RSD**
80%	169.1	0.00427	0.00426	99.95	Mean: 99.19% RSD: 0.64%
	168.5	0.00427	0.00422	98.91	
	168.7	0.00427	0.00420	98.40	
	169.2	0.00427	0.00424	99.34	
	168.4	0.00427	0.00421	98.67	
	168.7	0.00427	0.00426	99.88	
100%	211.3	0.00534	0.00530	99.29	Mean: 98.91% RSD: 0.32%
	212.4	0.00533	0.00526	98.56	
	212.3	0.00533	0.00527	98.78	
	211.8	0.00534	0.00528	98.85	
	212.3	0.00534	0.00530	99.32	
	211.8	0.00534	0.00527	98.68	
120%	254.6	0.00640	0.00629	98.29	Mean: 98.55% RSD: 0.35%
	253.1	0.00640	0.00630	98.47	
	253.8	0.00640	0.00631	98.61	
	254.2	0.00640	0.00628	98.11	
	253.9	0.00640	0.00632	98.79	
	254.7	0.00641	0.00634	99.06	
Average				98.89	
Overall RSD				0.51 %	

Linearity and Range:

Linearity Results:
Working standard Potency : 99.44%
Working standard ID : 09/WS-04

Concentration of standard in the working range i.e.50% to 150% of the nominal concentration in mg as follows**.**

Table- 3

Sr. No.	% Concentration	Concentration in mcg	Absorbance
1	50	2.5	0.251
2	80	4.0	0.387
3	100	5.0	0.490
4	120	6.0	0.584
5	150	7.5	0.731

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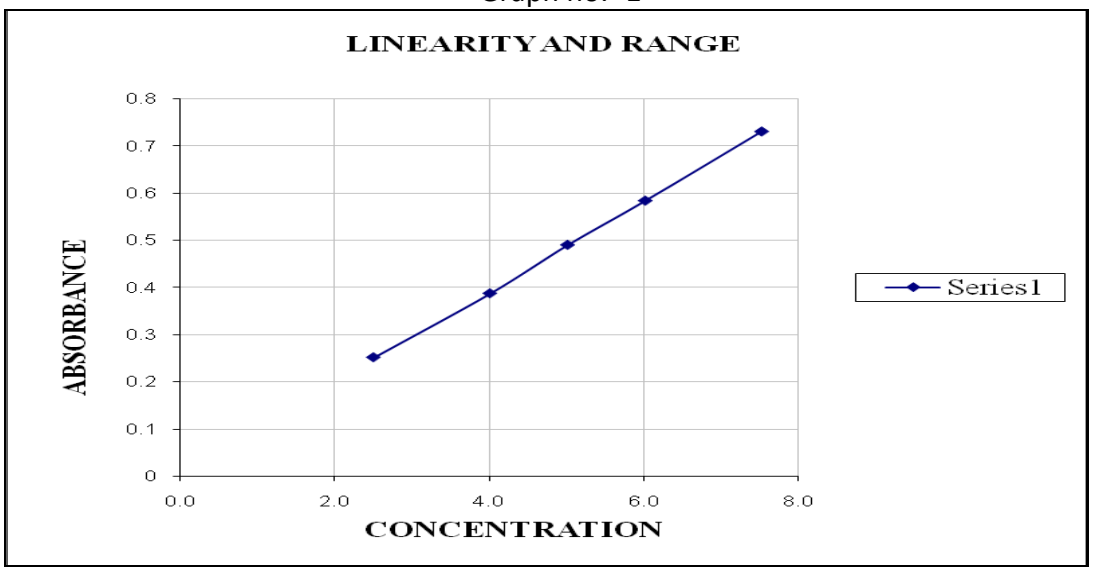
Table- 4

Linearity test Parameter			
Sr. No.	**Test**	**Result**	**Specification**
1	Conc. of standard Maximum	7.5	50 % higher than nominal concentration
2	Conc. of standard Minimum	2.5	50% Lower than the nominal concentration
3	Slope	0.0960	-
4	Intercept	0.0069	-
5	Correlation Coefficient (r)	0.9998	NLT 0.99

Linearity graphical presentation of standard Concentration vs. Absorbance

Graph no. -1



Precision or Reproducibility:

Precision test Results

Calculations: Calculate the Release of Norfloxacin as follows.

For Norfloxacin 400 mg tablet:

$$\text{mg / tab} = \frac{\text{Spl. Abs.}}{\text{Std. Abs.}} \times \frac{\text{Std. Wt.}}{100} \times \frac{1}{100} \times \frac{750}{1\text{ tab}} \times \frac{100}{1} \times \frac{P}{100}$$

$$\% \text{ Release} = \frac{\text{mg / tab}}{\text{Label Claim}}$$

Where,

Spl. Abs. : Sample Absorbance;

Std. Abs. : Standard Absorbance;

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Std. Wt. : Standard weight in mg

Analyst I, Day I: Norfloxacin Tablet 400mg

Table – 5

No. of Sample	Tablet No.	Sample Absorbance	% Release
1	1	0.462	88.61
2	2	0.467	89.57
3	3	0.459	88.04
4	4	0.470	90.15
5	5	0.466	89.38
6	6	0.462	88.61
		% Average	89.06
		% RSD	0.87

Ruggedness:

Ruggedness test Results

Calculations:

Calculate the Release of Norfloxacin as follows.

for Norfloxacin 400mg tablet:

$$mg / tab = \frac{Spl.\ Abs.}{Std.\ Abs.} \times \frac{Std.\ Wt.}{100} \times \frac{1}{100} \times \frac{750}{1\ tab.} \times \frac{100}{1} \times \frac{P}{100}$$

$$\% \text{ Release} = \frac{mg / tab}{\text{Label Claim}}$$

Where,

Spl. Abs. : Sample Absorbance;

Std. Abs. : Standard Absorbance;

Std. Wt. : Standard weight in mg;

Comparison between Analyst I & II: Norfloxacin Tablet 400mg

Table - 6

No. of Tablets	% Release	Analyst	Average / RSD
1	88.61	Analyst I	Average: 89.06% RSD: 0.87%
2	89.57		
3	88.04		
4	90.15		
5	89.38		
6	88.61		
7	87.17	Analyst II	Average: 88.18% RSD: 1.13%
8	88.69		
9	89.64		

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10	88.12		
11	88.50		
12	86.98		
Average %	88.62	% Difference (NMT 2.0%)	0.88%
% RSD	1.09		

SUMMARY & CONCLUSION

Summary:

The overall summary of Analytical method validation for Dissolution test of Norfloxacin Tablet 400mg is as follows.

Sr. No.	PARAMETER	SPECIFICATION LIMIT	OBSERVATION
1	Specificity	There shall not be any interference of the placebo at the maxima of active Norfloxacin.	No Interference
2	Accuracy	Overall RSD for 80%,100% and 120% shall be NMT 2% And release shall be between 98.0 to 102.0%	RSD :0.51 % % Release: 98.89 %
3	Linearity And Range	Correlation Coefficient- NLT 0.99	0.9998
4	Precision or Reproducibility	RSD for reproducibility shall be NMT 2%. And release shall not less than 80%(Q)	RSD :0.87 % % Release: 89.06%
5	Ruggedness	RSD for reproducibility NMT 5% and overall RSD shall be NMT 5%. Difference between two analyst shall be NMT 2%	RSD: 1.13% Overall RSD : 1.09% Difference : 0.88%

Conclusion

From the results it is observed that there is no any interference of the placebo found at the maxima of active Norfloxacin for Specificity. For Accuracy the Relative Standard Deviation for 80%, 100% and 120% concentrations are 0.64%, 0.32% and 0.35% and drug release are 99.19%, 98.91% and 98.55% respectively. Also the overall RSD is 0.51% and average release is 98.89% which show that the Accuracy (addition and recovery) of the test method is acceptable (Limit: RSD shall be NMT 2% and recovery shall be 98.0% to 102%). The Correlation Coefficient (r) for the data in the concentration range 2.5 to 7.5 is 0.9998 which is found well within the acceptable limit for Linearity and Range. For Precision or Reproducibility the %RSD for data obtained from Analyst I is 0.87% and average Release is 89.06%, which is found well within the acceptable limit. Ruggedness test the % RSD for data obtained from Analyst I is 0.87% & Analyst II is 1.13%, also the overall RSD for both the analyst is 1.09%, also the difference between two Analyst is 0.88% which is found well within the acceptable limit.

A series of tests were carried out to evaluate the performance parameters of the method as, Linearity, Precision, Ruggedness and Accuracy for the Dissolution of Norfloxacin in Norfloxacin tablet 400mg.Based on the results obtained by challenging the method with the above mentioned tests, the method stands valid for analytical purpose.

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