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Research

Designed method validation for new In-Vitro Dissolution by UV Visible Spectrophotometer for Metronidazole extended releases tablet by single chemical.

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Abstract

The present research work discusses the development of a UV spectrophotometric method for Metronidazole. Simple, accurate and cost efficient spectrophotometric method has been developed for the estimation of Metronidazole (MND) in Tablet dosage form. The optimal conditions for the drug analysis were established. The maximum wavelength (max) was found to be 278nm. The percentage recovery of the drug was calculated at 8 hours and obtained 99.4 % against criteria -Recovery should not be less than 95.0 and linearity observed as 0.9999. Validation was conducted in accordance with ICH guidelines, covering parameters such as linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ). The sample solution exhibited stability for up to 24 hours. The proposed method is deemed appropriate for the analysis of Metronidazole in tablet formulations for quality control applications.

Keywords: Metronidazole extended releases, UV Visible Spectrophotometer, Dissolution validation

INTRODUCTION

Designed method validation for new In-Vitro Dissolution by UV Visible Spectrophotometer for Metronidazole extended releases tablet by single chemical**.**Verification of the method used for the analysis of determination of dissolution of Metronidazole ER tablets 750 mg with the predetermine acceptance criteria 1st hour – between 25 to 45 %, 2nd hours- between 40 to 60 %, 8th hours= Not less than 80 %.Metronidazole, a BCS class I drug, could be waived based on the BCS principles, thus enabling in-Vitro dissolution data as a surrogate of BE study. However, the impact of dissolution profiles of metronidazole tablets on the in vivo performance has never been studied systematically.Product used for dissolution: FLAGYL® (metronidazole) extendedrelease tablets, 750mg. To reduce the development of drug-resistant bacteria and maintain the effectiveness of FLAGYL ER®, and other antibacterial drugs, FLAGYL ER® should be used only to treat or prevent infections that are proven or strongly suspected to be caused by bacteria.

MATERIALS AND METHODS

Equipment - Instrument –Glassware-Standard –Solvent-Chemicals Requirement

Equipment and Instrument: All equipment and instrument used during method validation shall be qualify, validate and within calibration and preventive maintenances validity.
UV –Visible Spectrophotometer: Model: ShimadzuInstrument number: QC/UV/030
pH meter: Model:Mettler-Instrument number : QC/pH/015
Analytical Balance Model: Mettler Instrument number : QC/BAL/026
Glassware: All Class A types glassware shall be used.Before used it shall be cleaned and dried as per validated and approved procedure.
Standards: Reference standards ofMetronidazole which has purity 99.99 %
Solvent and Chemicals Used during analysis: Hydrochloric acid HPLC grade, WaterHPLC grade, Metronidazole standard, Placebo of Metronidazole ER tablet 750 mg, Product used for dissolution: FLAGYL® (metronidazole) extended release tablets, 750 mg(Metronidazole ER tablet 750 mg).

Design Analytical method validation for dissolution by UV -Visible Spectrophotometer
Specificity andSystem Suitability: IdentificationBlank interference of the ExperimentSystem SuitabilityLinearity and RangePrecisionSystem PrecisionMethod PrecisionAccuracySolution StabilitySystem StabilitySolution stabilityRobustnessChange in wave lengthFilter variability.

Methodology of the Experiment
Chemical and Equipment: Chemicals: Hydrochloride acid HPLC grade, HPLC grade purified water, Dissolution parameters, Medium : 900 ml 0.1 N hydrochloric acid, Apparatus USP type ll paddle, Speed 50 RPM, Temperature 37ºC Sampling time 1,2 and 8 hours

Method for the Placebo preparation: Weight and transferred 300 mg of placebo in the each dissolution vessel containing 900ml dissolution medium. At the of the specified time point withdraw 10 ml of the sample solution though each dissolution vessel and replace with 10 ml of fresh dissolution medium. Filter the solution thought 0.45, micron membraneDilute 2.0 ml of the above filter solution to 100.0 ml with dissolution medium well,

Preparation of 0.1 NHydrochloride acid Dilute 85 ml of hydrochloric acid to 1000 ml with water and mix it well

Preparation of the standard: Accurateweight and transfer about 40 mg of standard Metronidazole into a 100 ml volumetric flask. Add about 60 ml of dissolution medium and sonic ate to dissolve. Diluteto volume with dissolution medium and mix it well
Dilute 2 ml of the above solution to 50.0 ml with dissolution medium and mix well

Preparation of Sample solution: Set the parameters of dissolution apparatus as mentioned above. Placeon tablet into each of the dissolution jar. At the end of the specified time point withdraw 10 ml of the sample solution from each dissolution vessel and replace with 10 ml of fresh dissolution medium. Filter the solution though 0.45 micronmembrane filter
Dilute 2.0 ml of the above solution to 100 ml with dissolution medium and mix it well

Validation Parameters: The main objective of method validation process is to prove that an analytical method is acceptable for its intended purpose. The necessity for laboratories to use fully validated methods is now universally accepted as a way to obtain reliable results. There are diverse documents for method validation including information about different performance parameters. The classical performance characteristics are accuracy, limit of detection, precision, recovery, robustness, ruggedness, selectivity, specificity and trueness. Unfortunately, contradictory information is normally present among the method validation documents used by laboratories.

Specificity and System Suitability: This test is performed for the identification of analyte and placebo interference

System Suitability: Measure the standard absorbance for ten replicates at about 278 nm 1 cm^2 cell.
Acceptance criteria: The % RSD of absorbance should NMT 2.0

Identification

Results of Identification :		
Type of Spectrum	**Wave length maxima**	**Absorbance**
Standard	276.50	0.621
Sample	276.50	0.583
Conclusion : The spectrum of standard sample should be comparable with respect to wavelength. Hence method is specific.		

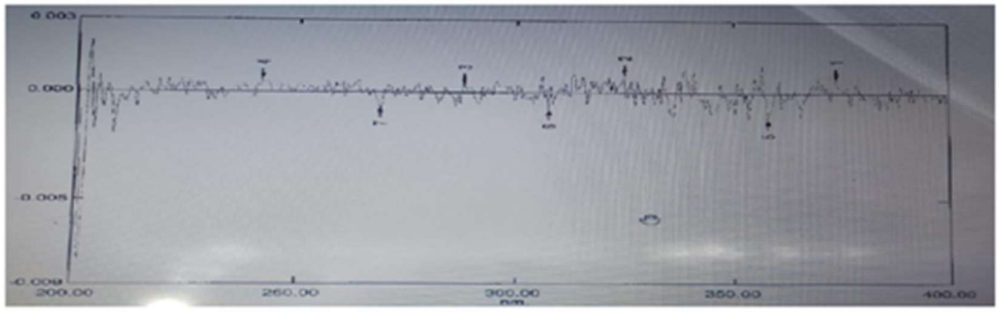
Blank and Placebo interference

Results of Blank and Placebo interference		
Sr.no	**% Blank Interference**	**% Placebo Interference**
1	0.00	0.00
2	0.00	0.00
3	0.00	0.00
Maxima	0.00	0.00
Conclusion : The spectrum of standard sample should be comparable with respect to wavelength. No interference. Hence method is specific.		

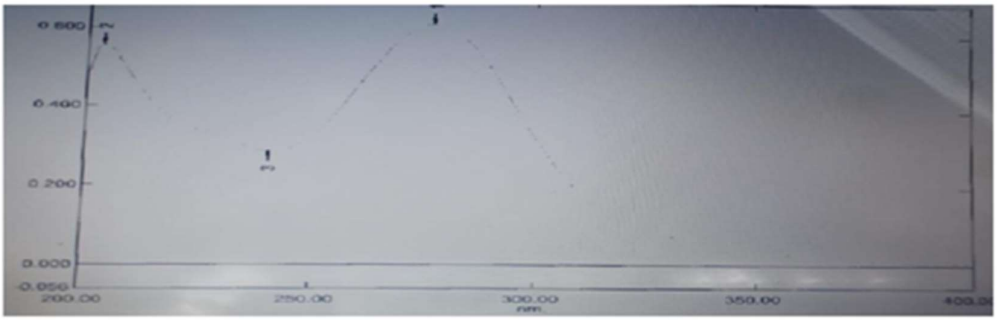
System Suitability

Result of System Suitability	
Replicate	**Absorbance**
1	0609
2	0.608
3	0.609
4	0.608
5	0.609
6	0.608
7	0.607
8	0.608
9	0.607
10	0.608
Mean	0.608
SD	0.0007
% RSD	0.12
Acceptance criteria: The % RSD of absorbance should NMT 2.0. Hence method is specific.	

Wavelength range 200 to 400 Placebo



Wavelength range 200 to 400 Standard

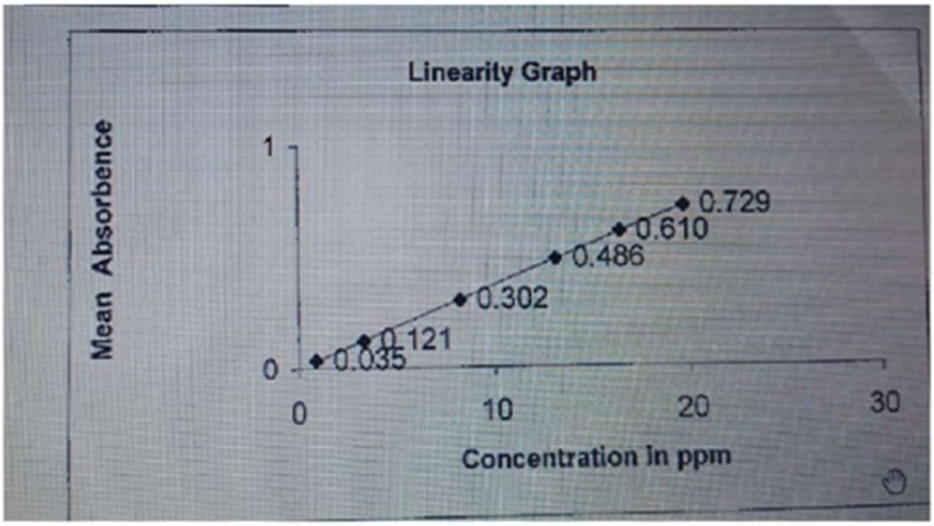


Summary of Specificity and System Suitability				
Specificity	**Validation parameters**	**Results**		**Acceptance criteria:**
		Wave length		
	Standard Wavelength maxima	276.50	Absorbance 0.621	The spectrum of standard sample should be comparable with respect to wavelength
	Sample Wavelength maxima	276.50	Absorbance 0.583	
	Blank Interference	0.00		The Maximum % interference due to blank and placebo should be NMT 2.0
	Placebo	0.00		
System Suitability	% RSD	0.12		The % RSD of absorbance should NMT 2.0

Linearity

Linearity of analyte from 5 % of lower specification to 120 % of higher specification level Perform the linearity of the analyte from 5% to 120% target concentration -1 ppm to 20 ppm by taking minimum five concentration levels and the measures the absorbance at 278 nm in 1cm^{2}cell.For 5 % and 120% of target concentration measure the absorbance 6 times and for the other levels in duplicate.Plot graph of concentration against the absorbance and calculate the linearity regression coefficient % Y intercept, residual sum of squares and % RSD of absorbances for 5 % and 120% of the target concentration.Correlation coefficient should be less than 0.999, % Y intercept should be between ± 2.0, % RSD of absorbance at 5% level and 120% level should be NMT 2.0

Results of linearity :					
Linearity Levels	**Concentration in ppm**	**Mean Absorbance**	**% RSD**	**Statistical Analysis**	
L1	0.877	0.35.121	1.16	R2	1.00000
L2	3.288	0.121	--	Slope	0.033690
L3	8.220	0.302	--	Y intercept	0.001
L4	13.152	0.486	--	% Y- intercept	0.16
L5	6.440	0.610	--	Correlation coefficient	0.99998
L6	19.728	0.729	0.23	Residual sum of squares	0.0020
Conclusion: response is linear over the concentration range from 5 % to 120 % of target concentration.					



Summary of Linearity			
	Validation parameters	**Results**	**Acceptance criteria**
Linearity	R2	1.00000	NA
	Slope	0.033690	NA
	Y intercept	0.001	NA
	% Y Intercept	0.16	% Y intercept should be between ± 2.0
	Correlation Coefficient	0.99998	Correlation coefficient should be less than 0.999
	Residual sum of squares	0.0020	NA

Precision: In the precision methods at least six determination method precision shall be demonstrated
System Precision: Prepare standard solution as per the test method and measure the absorbance five times at 278 nm using 1cm^{2}cell.% RSD for five absorbance's should be NMT 2.0.

Result of System Precision			
Sr.No	**Standard absorbance**		
	1 sthour	2nd Hour	8 thhour
1	0.613	0.607	0.615
2	0.612	0.606	0.614
3	0.614	0.607	0.615
4	0.613	0.607	0.615
5	0.614	0.607	0.615
Mean	0.613	0.607	0.615
SD	0.0007	0.0004	0.0004
% RSD	0.11	0.07	0.07
Acceptance criteria: % RSD for five absorbance's should be NMT 2.0			

Method Precision: Prepare six sample preparations as per test method for each time point and measure the absorbance at 278 nm using 1cm^{2}cell. And calculate percentage of dissolution.The % RSD of % dissolution from six samples for each time point should be NMT 5.0.

Result of Method Precision			
Sr.No	**Standard absorbance**		
	1 sthour	2nd Hour	8 thhour
1	32.5	48.2	93.6
2	34.1	49.8	90.6
3	32.4	48.8	90.7

4	34.4	49.7	93.1
5	33.0	48.4	90.9
6	32.5	48.6	92.0
Mean	33.2	48.6	92.0
SD	0.88	0.70	1.40
% RSD	2.65	1.43	1.52

Acceptance criteria: % RSD for five absorbance's should be NMT 2.0

Summary of Precision

	Validation parameters	**Results**		**Acceptance criteria:**
System Precision;	% RSD	1st hour	0.11	% RSD for five absorbance's should be NMT 2.0
		2nd hour	0.07	
		8th hour	0.07	
Method Precision	% RSD	1st hour	2.65	The % RSD of % dissolution from six samples for each time point should be NMT 5.0
		2nd hour	1.43	
		8th hour	1.52	

System Precision

Prepare standard solution as per the test method and measure the absorbance five times at 278 nm using 1cm^{2}cell.% RSD for five absorbance's should be NMT 2.0

Result of System Precision			
Sr.No	**Standard absorbance**		
	1 sthour	2nd Hour	8 thhour
1	0.617	0.617	0.620
2	0.615	0.617	0.619
3	0.616	0.617	0.620
4	0.616	0.618	0.620
5	0.617	0.617	0.619
Mean	0.616	0.617	0.620
SD	0.0005	0.0004	0.0004
% RSD	0.08	0.06	0.06
% RSD for five absorbance's should be NMT 2.0			

Method Precision: Dissolution was performed with sic tablets as per the tedtmethos and measured the absorbance of each solution by using UV spectrophotometer and calculation the % dissolution, Prepare six sample preparations as per test method for each time point and measure the absorbance at 278 nm using 1cm^{2}cell. And calculate percentage of dissolution.The % RSD of % dissolution from six samples for each time point should be NMT 5.0.The overall % RSD of % dissolution from precision study and intermediate precision study for each time point should be NMT 5.0

Results of Method Precision			
Sr.No	**Standard absorbance**		
	1 sthour	2nd Hour	8 thhour
1	34.2	50.5	92.9
2	32.8	47.7	94.0
3	35.3	49.1	92.1
4	32.8	49.6	92.9
5	33.3	47.2	94.1
6	35.0	48.2	91.3
Mean	33.9	48.7	92.6
SD	1.10	1.24	1.08
% RSD	3.24	2.55	1.16
The % RSD of % dissolution from six samples for each time point should be NMT 5.0			

Over all % Dissolution statistics for 1 hours

Sample	% Dissolution for 1 hours (Overall Statistics)	
	Set 1	Set 2

1	32.5	34.2
2	34.1	32.8
3	32.4	35.3
4	34.4	32.8
5	33.0	33.3
6	32.5	35.0
Mean	33.2	33.9
SD	0.88	1.10
% RSD	2.65	3.24
Over all Mean	33.5	
Over All SD	1.03	
Over All % RSD	3.07	
Analyst	ABC	EFG
Set	1	2
Day	1	2
Instrument	QC-UV-001	QC-UV-004

Over all % Dissolution statistics for 2nd hours

Sample	% Dissolution for 1 hours (Overall Statistics)	
	Set 1	Set 2
1	48.2	50.5
2	49.8	47.7
3	48.8	49.1
4	49.7	49.6
5	48.4	47.2
6	48.6	48.2
Mean	48.6	48.7
SD	0.70	1.24
% RSD	1.43	2.55
Over all Mean	48.8	
Over All SD	0.96	
Over All % RSD	1.97	
Analyst	ABC	EFG
Set	1	2
Day	1	2
Instrument	QC-UV-001	QC-UV-004

Over all % Dissolution statistics for 8th hours

Sample	% Dissolution for 1 hours (Overall Statistics)	
	Set 1	Set 2
1	93.6	92.9
2	90.6	94.0
3	90.7	92.1
4	93.1	92.9
5	90.9	94.1
6	92.0	91.3
Mean	92.0	92.6
SD	1.40	1.08
% RSD	1.52	1.16
Over all Mean	92.4	
Over All SD	1.28	
Over All % RSD	1.39	
Analyst	ABC	EFG
Set	1	2
Day	1	2
Instrument	QC-UV-001	QC-UV-004

Summary Precision

	Validation parameters	Results		Acceptance criteria:
System Precision :	% RSD	1st hour	0.08	% RSD for five absorbance's should be NMT 2.0
		2nd hour	0.06	
		8th hour	0.06	
Method Precision	% RSD	1st hour	3.24	The % RSD of % dissolution from six samples for each time point should be NMT 5.0
		2nd hour	2.55	
		8th hour	1.16	
	% RSD-Over All	1st hour	3.07	The % RSD of % dissolution from precision study and intermediate precision study for each time point should be NMT 5.
		2nd hour	1.97	
		8th hour	1.39	
Conclusion: The test shoes the method is rugged				

Accuracy: The accuracy shall be performed by placebo spiked with known amount of analysts by using at least 3 replicates of 3 test concentrations levels.

System Precision: Prepare standard solution as per the test method and measure the absorbance five times at 278 nm using 1cm^{2}cell. % RSD for five absorbances should be NMT 2.0

Accuracy: Prepare sample solution by spiking the analyte to the placebo at known concentration Level ranging from 5 % to 120 % of target concentration by using at least three replicate of minimum three concentration levels and measure the absorbance.% Recovery should not be less than 95.0

Accuracy Results

Accuracy level		Amount added in mg	Amount found in mg	% Recovery	Statistical analysis	
Level-1	Sample -1	38.59	38.31	99.3	Mean	99.8
	Sample -2	38.57	38.31	99.3		
	Sample -3	39.16	39.51	100.9		
Level-2	Sample -1	151.00	149.64	99.1	Mean	99.0
	Sample -2	151.05	148.45	99.0		
	Sample -3	150.28	149.64	98.8		
Level-3	Sample -1	376.97	374.71	99.6	Mean	99.2
	Sample -2	376.39	372.31	99.2		
	Sample -3	375.26	372.31	99.3		
Level-4	Sample -1	598.73	594.98	99.4	Mean	99.4
	Sample -2	5.99.17	596.18	99.2		
	Sample -3	598.33	593.78	99.4		
Level-5	Sample -1	751.38	745.82	99.3	Mean	99.4
	Sample -2	750.28	747.02	99.6		
	Sample -3	750.02	742.82	99.4		
Level-6	Sample -1	895.62	893.07	99.7	Mean	99.6
	Sample -2	896.13	891.87	99.5		
	Sample -3	895.33	891.87	99.6		
Over all Statistical analysis						
Mean	99.4	SD	0.46		% RSD	0.46
The recovery results indicates that the test method has an acceptable level of Accuracy						

Accuracy Summary

	Validation parameters	Results	Acceptance criteria:
Accuracy	% Mean recovery	99.4	% Recovery should not be less than 95.0
Conclusion: The recovery results indicates that the test method has anacceptable level of accuracy			

Range

Evaluate the range of methods using the data from linearity, Precision and accuracy studies

Range		
	Validation parameters	**Results**	**Acceptance criteria:**
Range	5 % to 120 % of target concentration		
Conclusion: Range of the analytical method can be obtained from linearity, precision and accuracy data.Report the range in % with respect to sample concentration			

Solution Stability

System Precision: Prepare standard solution as per the test method and measure the absorbance five times at 278 nm using 1cm^{2}cell.% RSD for five absorbance's should be NMT 2.0

Carryout the solution stability for standard and 8th hour time point sample solutions and measure the absorbance at 278 nm in 1 cm2 cell at regular interval 2 hours, 4 hours, 8 hours, 12 hours , 24 hours along with freshly prepared standard.For standard the % difference of % Assay for initial standard to standard at regular intervals should be NMT 2.0. For sample the % difference of % dissolution for initial sample to sample at regular intervals should be NMT 2.0

Results of solution stability		
Time in hours	**% Assay**	**% Difference**
Standards solution		
Initial	99.8	-
2 hours	99.6	0.2
4 hours	100.0	0.2
8 hours	99.1	0.7
12 hours	99.1	0.7
24 hours	99.4	0.4

Results of solution stability		
Time in hours	**% Dissolution**	**% Difference**
Sample solution		
Initial	92.3	-
2 hours	92.6	0.3
4 hours	93.2	0.1
8 hours	92.6	0.3
12 hours	93.3	1.1
24 hours	93.2	1.0

Summary Solution Stability			
	Validation parameters	**Results**	**Acceptance criteria:**
Standard solution	Standard solution is stable up to 24 hours on the bench		-----
Sample solution	Samplessolution is stable up to 24 hours on the bench		------

Robustness

Change in wavelength: Prepare standard solution as per the test method and measure the absorbance by alternating by 278± 5 nm (273 nm and 283 nm). System suitability should pass as per test method at variable conditions. The % difference of % dissolution compared from 278± 5 nm should be NMT 5.0

	Results of Change in wavelength				
Sr.no	**278nm-273nm-283nm**				
	% Dissolution			**Difference**	
	At 278nm	**273nm-**	**283nm**	**At 278nm Vs 273nm**	**At 278nm Vs 283 nm**
1	94.0	93.8	93.7	0.2	0.3
2	93.1	92.4	92.7	0.8	0.4

3	92.9	92.7	92.9	0.2	0.0
The % difference of % dissolution compared from 278± 5 nm should be NMT 5.					

Filter Variability: Prepare 3 samples solution for 8thhours time point as per the test methods. Centrifuge one portion of sample solution and filter the other portion of sample solution through at least two types of filters (PVDF and nylon 66 filter). Note: Sample preparations in precision study can be used in filter variability. For % difference of % dissolved compared to centrifuge to the filtered samples s should be NMT 5.0

Results of Filter Variability					
Sr.no	**Centrifuged -PVDF -nylon 66 filter**				
	% Dissolution			**Difference**	
	Centrifuged	**Nylon 66**	**PVDF**	**Nylon 66**	**PVDF**
1	92	92.1	91.7	91.7	0.3
2	92.3	93.2	91.5	91.5	0.9
3	93.1	92.4	92.4	92.5	0.8
For % difference of % dissolved compared to centrifuge to the filtered samples s should be NMT 5.0					

Robustness

	Validation parameters	**Results**	**Acceptance criteria:**
Filter Variability	Maximum % Difference (Centrifuged Vs PVDF)	0.9	For % difference of % dissolved compared to centrifuge to the filtered samples s should be NMT 5.0
	Maximum % Difference (Centrifuged Vs Nylon 66)	1.0	
Change in Wave length	Maximum % Difference (278 Vs 273)	0.8	The % difference of % dissolution compared from 278± 5nm should be NMT 5.0
	Maximum % Difference (278 Vs 283)	0.4	

CONCLUSION

This study describes a UV method that has been used to assess metronidazole tablets and related validation parameters using various solvent systems of these water: hydrochloride for combination mixture of metronidazole tablets have been shown the possible better findings out this assay method. This proposed solvent system meets all of the method validation criteria, such as linearity, accuracy and precision.

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