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Research

Experimental design for novel in-vitro dissolution method validation for lamivudine-150mg and zidovudine-300mg film-coated tablets

K. Aishwarya*, K. Vamshikrishna, Mohd Omar, R.Rajareddy

Arya College of Pharmacy Sangareddy, Affiliated to Osmania University, Hyderabad, Telangana 502285

Corresponding Author:K. Aishwarya
Email: aishwaryakada@gmail.com

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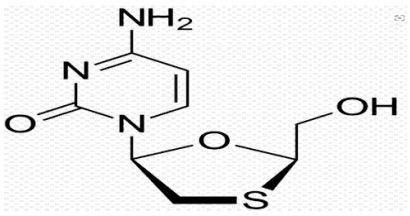
Abstract

The present study objective is Experimental Design for novel in-vitro Dissolution method validation for Lamivudine150 and Zidovudine 300 mg film-coated tablets and its provide the direction to design and conduct performance test for the same and established documentary evidence through the test method defined in the study , its helps to demonstrate that the chromatographic analytical methods for determination of dissolution in Lamivudine/Zidovudine 150 mg/300 mg film-coated tablets Films coating tablet will yield consistent, reliable and reproducible results within in the pre-determined acceptance criteriaThe active ingredient Lamivudine and Zidovudine and Inactive ingredients include microcrystalline cellulose, sodium starch glycolate, colloidal silicon dioxide, povidone, magnesium stearate, and opadry white (composed of Hydroxy Propyl methylcellulose 2910/Hypromellose 5cP, Titanium dioxide, and Polyethylene glycol 400).

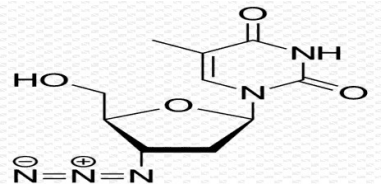
Keywords: Experimental design, Lamivudine, Zidovudine, HPMC, PEG 400.

INTRODUCTION[1,2]

Lamivudine is a prescription nucleoside reverse transcriptase inhibitor (NRTI) that is used in combination with other drugs as antiviral treatment for human immunodeficiency virus type-1 (HIV-1) and as aimmunotherapy for hepatitis B virus (HBV)



Zidovudine is used along with other medications to treat human immunodeficiency virus (HIV) **infection**. Zidovudine is given to HIV-positive pregnant women to reduce the chance of passing the infection to the baby. Zidovudine is in a class of medications called nucleoside reverse transcriptase inhibitors (NRTIs).



Experimental design
To conduct the method validation of chromatographic method for the dissolution of Zidovudine USP 300 mg and Lamivudine USP in Specification not less than 80% in 30 minutes.Analytical method validation for dissolution test are carried out by considering following analytical parameter: Specificity and System Suitability, Linearity and RangePrecision, System Precision,Method Precision, Intermediate Precision- Ruggedness, Analyst to Analyst, Stability of standard and analyte solutionAccuracyRobustnessSolution Stability

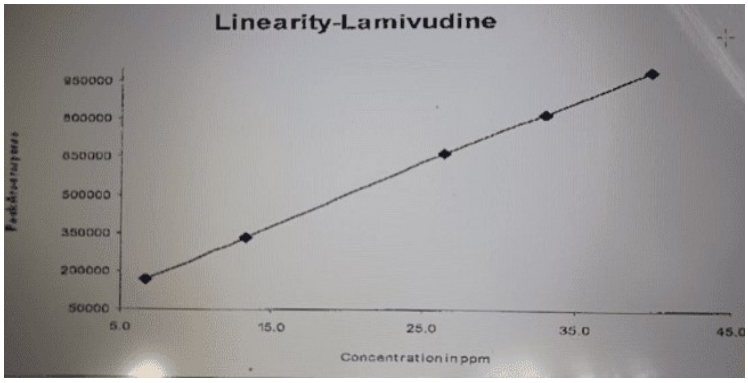
Equipment and Instrument: Dissolution Apparatus*: Model:* Lab India with Auto samplerInstrument *number:* QC/DISO/*004**HPLC**Model:* WatersInstrument *number:* QC/HPLC/023 **Analytical *Balance****Model:* MettlerInstrument *number:* QC/BAL/002*,* **Standards*:*** *Lamivudine* USP and Zidovudine *USP which* has purity 99.99 %**Solvent and Chemicals Used during analysis**HPLC and analytical grade materialsAcetonitrileAmmonia AcetatePurified water

METHODOLOGY

Standard Preparation: Weight accurately about 25 mg of Lamivudine reference standard and 32.5 mg of Zidovudine reference standard into a 50 ml volumetric flask and dissolves with 15ml diluents. Sonicate at 37.0 ± 0.5 o C for 10 minute. Dilute to the mark with diluents. Dilute 10 ml of this solution to 100ml diluents Inject 10 µl the standard solution in the range of 20 % and 120 % 6 times and other levels in duplicate. Plot a graph of concentration against the peak response and calculate the linearity regression coefficient, % Y-Intercept, residual sum of squares and % RSD of 20% and 120 % level for peak area responses.
Acceptances Criteria: Correlation coefficient shall not be less than 0.99% Y intercept shall be between ± 2.0, % RSD of peak area response area Reponses of 20% and 120% level shall be NMT 2.0

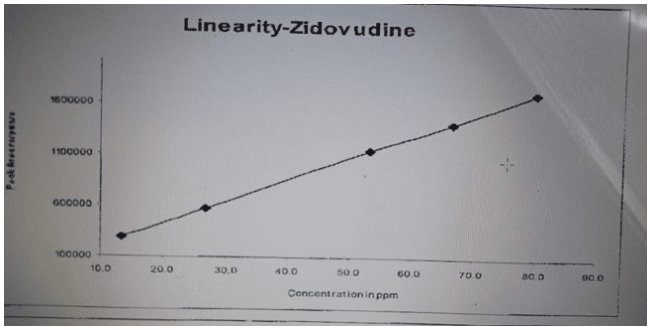
Lamivudine					
Level	**Concentration-ppm**	**Area Average**	**% RSD**	**Statistical Analysis**	
L1	6.7	165164	0.78	SLOPE	24087.0520
				Y-Intercept	6310.155
L2	13.3	323529	NA	% -Intercept	0.78
L3	26.7	656227	NA	Correlation Coefficient	0.99999
L4	33.3	804754	NA	Residual sum of square	5282.9581
L5	40.0	968168	0.15	r2	0.9998

Lamivudine Linearity plot – Concentration versus Peak area Response



Zidovudine					
Level	**Concentration-ppm**	**Area Average**	**% RSD**	**Statistical Analysis**	
L1	13.3	259122	0.05	SLOPE	216.583
				Y-Intercept	7503.581
L2	26.6	573999	NA	% -Intercept	0.53
L3	53.3	1159719	NA	Correlation Coefficient	.99999
L4	66.6	1421669	NA	Residual sum of square	9934.0390
L5	79.9	1711011	0.13	r2	0.9998

Zidovudine Linearity plot – Concentration versus Peak area Response



Acceptances criteria	
System Suitability	- The column efficiency as determine from Lamivudine USP and Zidovudine USP peaks are not less than 2500 theoretical plates. - The tailing factor for the same peaks are not more than 2.0
System Precision	- RSD for the peak areas of the five replicate injection s of Lamivudine and Zidovudine peaks are not more tha 2.0%
Method Precision	- The % RSD of % Dissolution from six samples should not be less NMT 5.0

Evaluation of System Suitability

Conclusion :System Suitability		
Compound	Lamivudine USP	Zidovudine
Column Efficiency	4131	2641
Tailing Factor	1.38	1.06

System Preparation- Standard Solution

Sr.No	Lamivudine		Zidovudine	
	Retention time in minute	Peak Area	Retention time in minute	Peak Area
1	2.934	806018	4.268	1421605
2	2.931	804450	4.272	1422594
3	2.933	805290	4.272	1421750
4	2.934	805213	4.267	1422237
5	2.935	804559	4.2.63	1419986
Average	2.934	805106	4.267	1421459
% RSD	0.03	0.08	0.09	1421605
Conclusion : Test results are showing that the system is precise				

Method Precision

Sample	% w/w of	
	Lamivudine	Zidovudine
1	102.82	105.49
2	95.8397.23	98.24
3	97.69	96.25
4	95.83	100.96
5	98.98	91.40
6	90.26	98.6
Average	97.1	98.6
% RSD	4.25	4.78
Conclusion : Test results are showing that the method is precise		

Standard Preparation : System Suitability
The column efficiency as determine from Lamivudine USP and Zidovudine USP peaks are not less than 2500 theoretical plates.
The tailing factor for the same peaks are not more than 2.0

System Precision: RSD for the peak areas of the five replicate injection s of Lamivudine and Zidovudine peaks are not more tha 2.0%

Method Precision The % RSD of % Dissolution from six samples should not be less NMT 5.0

Evaluation of System Suitability

Conclusion :System Suitability		
Compound	Lamivudine USP	Zidovudine
Column Efficiency	5925	2741
Tailing Factor	0.08	0.07

System Precision-Standard Solution

Concentration	Lamivudine		Zidovudine	
	Retention time in minute	Peak Area	Retention time in minute	Peak Area
1	2.8372	811322	4.107	1425117
2	2.848	812917	4.128	1425687
3	2.837	811148	4.117	1425996
4	2.837	816828	4.117	1434331
5	2.837	813137	4.117	1427644
Average	2.839	813070	4.117	1427767
% RSD	0.17	0.28	0.18	0.07
Conclusion : Test results are showing that the System is precise				

Overall Statistical Analysis

% w/w of	Mean	SD	% RSD
Lamivudine	99.4	4.143	4.17
Zidovudine	100.7	4.352	4.32
Set		Analyst 1	Analyst 2
Column		QCD-115	QCD-120
Equipment ID		AD051	AD064
Conclusion : Test results are showing that the method is Rugged			

Lamivudine					
Concentration	**Amount added (ppm)**	**Amount added (ppm)**	**% Recovery**	**Statistical Analysis**	
Sample-1/20%	32.51	32.51	100.0	Mean	101.5
Sample-2/20%	32.12	32.54	101.3		
Sample-3/20%	31.45	32.44	103.1		
Sample-1/100%	150.80	154.39	102.4	Mean	102.2
Sample-2/100%	150.65	153.87	102.1		
Sample-3/100%	150.84	154.04	102.1		
Sample-1/120%	192.50	200.66	104.2	Mean	103.6
Sample-2/120%	187.48	192.58	102.7		
Sample-3/120%	190.54	197.84	103.8		

Zidovudine					
Concentration	**Amount added (ppm)**	**Amount added (ppm)**	**% Recovery**	**Statistical Analysis**	
Sample-1/20%	62.35	65.30	103.1	Mean	102.9
Sample-2/20%	62.58	64.38	102.9		
Sample-3/20%	62.46	64.06	102.6		
Sample-1/100%	299.90	306.95	102.4	Mean	102.1
Sample-2/100%	299.86	306.04	102.1		
Sample-3/100%	300.02	305.63	101.9		
Sample-1/120%	380.60	397.38	104.4	Mean	103.1
Sample-2/120%	375.57	382.63	101.9		
Sample-3/120%	372.64	390.38	104.8		

The recovery results indicating that the test has an acceptable level of accuracy for determination of Lamivudine and Zidovudine in film coated tablets from 20 % level to 120 % of target Concentration.

Range

Method Procedure

Range of analytical method can be obtained from linearity, precision and accuracy data. Report the range in % with respect to specification.From the above data from linearity, precision and accuracy data, it is concluded that the range of analytical methods for determination of dissolution in film coating tablet is from 20% level to 120 % of target concentration.

Robustness

validate the analytical procedure capability to remain un affected by small but deliberate variation in method parameters and provides indicates of its reliability during normal usage. Evaluate the analytical methods robustness for the following typical variation from set procedure. Inject 10µl of standard solution five times in the chromatographic system and record the % RSD of peaks areas as per procedure.Inject 10µl of test solution three times in the chromatographic system and record the % RSD of peaks areas as per procedure

Alter the below mentioned chromatographic condition

Influence of flow rate variations

From 1.0 ml/min to 0.9 ml/ min

From 1.0 ml/min to 1.1 ml/ min

Influence of variation of temperature

From 25 ºC to 20ºC

From 25 ºC to 30ºC

Acceptances criteria :	
System Suitability	- The column efficiency as determine from Lamivudine USP and Zidovudine USP peaks are not less than 2500 theoretical plates. - The tailing factor for the same peaks are not more than 2.0 - The % RSD of % Dissolution from five injection of Lamivudine USP and Zidovudine USP should not be less NMT 5.
System Precision	- The overall % RSA of % Dissolution from Method precision study and robustness study together should be NMT 5.0

Standard Solution

System Suitability parameter		**Theoretical Plate**		**Tailing Factor**		**% RSD of Peak area**	
		Lamivudine	**Zidovudine**	**Lamivudine**	**Zidovudine**	**Lamivudine**	**Zidovudine**
Flow change	09ml	5030	2659	1.75	1.16	0.12	0.12
	1.1 ml	4223	2956	1.71	1.18	0.08	0.03
Temperature change at 20ºC		4235	3256	1.76	1.18	0.26	0.10
Temperature change at 30ºC		4405	2756	1.74	1.22	0.11	0.05
Mobile Phase Variation	Buffer (775) : Acetonitrile(275)	4810	2659	1.76	1.14	0.04	0.11
	Buffer (725) : Acetonitrile(275)	4335	2636	1.74	1.21	0.02	0.13

Sample Preparation

System Suitability parameter		**% RSD**	
		Lamivudine	**Zidovudine**
Flow change	09ml	2.25	2.46
	1.1 ml	2.34	2.49
Temperature change at 20ºC		2.29	2.40
Temperature change at 30ºC		2.41	2.52
Mobile Phase Variation	Buffer (775) : Acetonitrile(275)	2.36	2.25
	Buffer (725) : Acetonitrile(275)	2.53	2.64

Overall Statistical Analysis

System Suitability parameter		**% RSD**	
		Lamivudine	**Zidovudine**
Flow change	09ml	4.27	4.73
	1.1 ml	4.37	4.84
Temperature change at 20ºC		4.23	4.64
Temperature change at 30ºC		4.46	4.74
Mobile Phase Variation	Buffer (775) : Acetonitrile(275)	4.43	4.89
	Buffer (725) : Acetonitrile(275)	4.51	4.82

Solution stability

Demonstrate the solution stability of the standard and sample solution by injecting in regular interval

Methodology

Inject 10µl of standard solution five times in the chromatographic system and record the % RSD of peaks areas as per procedure, Separately inject 10µl of test solution three times in the chromatographic system and record the % RSD of peaks areas as per procedure-One timeInject 10µl of freshly standard solution one time along with

exiting standard and test sample solution, one time at regular interval 6 Hrs. in the chromatographic system and record the % RSD of peaks areas as per procedureSame procedure is applicable 6 hrs,12 hourd,24 hours and 48 hrs.

Acceptances criteria
- The column efficiency as determine from Lamivudine USP and Zidovudine USP peaks are not less than 2500 theoretical plates. - The tailing factor for the same peaks are not more than 2.0 - The % RSD of % Dissolution from five injection of Lamivudine USP and Zidovudine USP should not be less NMT 2.0 - For standard the % different of assay for initial standard to standard to standard at regular intervals shall be NMT 2.0 - For sample the % different of assay for initial standard to standard to standard at regular intervals shall be NMT 2.0

Standard Solution

System Suitability parameter	**% Difference**	
	Lamivudine	**Zidovudine**
Initial	--	--
About 6 hours	1.08	0.79
About 12 hours	0.57	0.57
About 18 hours	0.90	1.46
About24 hours	0.34	0.86
About 48 hours	0.04	1.02

Sample Solution

System Suitability parameter	**% Difference**	
	Lamivudine	**Zidovudine**
Initial	--	--
About 6 hours	1.30	0.34
About 12 hours	0.09	0.94
About 18 hours	0.66	1.90
About24 hours	0.68	1.37
About 48 hours	0.35	0.63

From the above data it is concluded that, the sample and standard are stable for 48 hours on the analysis bench top

Summary and conclusion: The test method is validated and meets the predetermine acceptance *criteria and used for dissolution test for routine procedure.*

Validation Data		**Results**	**Acceptances criteria**
SPECIFICITY			
Identification	Standard Solution		RT obtained with the standard solution should be comparable with the sample solution
	Lamivudine	2.934	
	Zidovudine	4.263	
	Sample Solution		
	Lamivudine	2.934	
	Zidovudine	4.263	
LINEARITY			
Linearity	Lamivudine	0.9999	Correlation coefficient shall be not less than 0.99
	Zidovudine	0.9999	
PRECISION			
System Precision	% RSD		% RSD of Peak area is not more than 2.0
	Lamivudine	Zidovudine	
	0.08	0.07	
Method Precision	% RSD		
	Lamivudine	4.25	% RSD should not be more than 5.0
	Zidovudine	4.78	

Validation Data		Results	Acceptances criteria
Intermediate Precision- Ruggedness			
System Precision	% RSD	% RSD	% RSD of Peak area is not more than 5.0
	Lamivudine	Zidovudine	
	0.28	0.27	
Method Precision	% RSD		
	Lamivudine	2.7	% RSD should not be more than 5.0
	Zidovudine	2.80	
Over all	Lamivudine	4.17	
	Zidovudine	4.32	
Accuracy			
20%	Lamivudine	101.5	The % recovery of accuracy level should be less than 95.0
	Zidovudine	102.9	
100%	Lamivudine	102.2	
	Zidovudine	102.1	
120%	Lamivudine	103.6	
	Zidovudine	103.7	
Range			
Range	The range is 20% to 120 % of specification		----
Robustness			
Robustness	Flow Variation±10%	Complies	System Suitability shall pass as per test method The overall % RSD of dissolution from method precession study and robustness together shall be more than 5.0
	Temperature Variation ±5 ºC	Complies	
	Mobile Phase ±20%	Complies	
Solution Stability			
Standard Solution	Stable for 48 hours		---
Sample Solution			---

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