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R E S E A R C H A R T I C L E

Analytical Method Development and Validation for Lamivudine and Zidovudine Combine Pharmaceutical Dosage Forms by RP-HPLC

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A B S T R A C T

A new method was established for simultaneous estimation of Lamivudine and Zidovudine by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Lamivudine and Zidovudine by using Agilent C18 column (4.6×150mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol:Acetonitrile, detection wavelength was 254 nm. The instrument used was Shimadzu, model No. SPD-20MA LC+20AD, Software- LC-20 Solution. The retention times were found to be 2.335 mins and 3.400 mins. The % purity of Lamivudine and Zidovudine was found to be 99.85% and 100.34% respectively. The system suitability parameters for Lamivudine and Zidovudine such as theoretical plates and tailing factor were found to be 2284, 1.7 and 2886 and 1.7, the resolution was found to be 5.4. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Lamivudine and Zidovudine was found in concentration range of 10µg-50µg and 20µg-100µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.10 and 0.1, % RSD for intermediate precision was 0.4 and 0.3 respectively. The precision study was precision, robustness and repeatabilty.LOD value was 2.17 and 0.0372 and LOQ value was 6.60 and 0.1125 respectively.

Keywords: Agilent C18, Lamivudine and Zidovudine RP-HPLC

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C O N T E N T S

1. Introduction

Lamivudine, commonly called 3TC, is an antiretroviral medication used to prevent and treat HIV/AIDS. It is also used to treat chronic hepatitis B when other options are not possible. It is effective against both HIV-1 and HIV-2. It is typically used in combination with other antiretroviral such as zidovudine and abacavir.

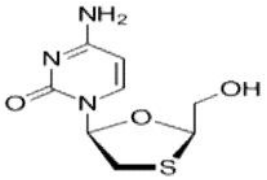

Fig 1: Chemical structure of Lamivudine

Zidovudine (ZDV), also known as azidothymidine (AZT), is an antiretroviral medication used to prevent and treat HIV/AIDS. It is generally recommended for use with other antiretrovirals.

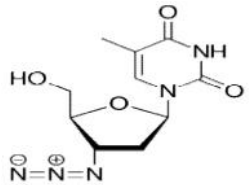

Fig 2: Chemical Structure of Zidovudine

2. Materials and Methods

Instrumentation

HPLC Shimadzu, model No. SPD-20MA LC+20AD, Software- LC-20 Solution, U.V double beam spectrometer UV 3000+ U.V win software Lab India Digital weighing balance(sensitivity 5mg) pH meter Sonicator.

Materials:

Lamivudine and Zidovudine, Ortho phosphoric acid, KH_2PO_4, K_2HPO_4, Acetonitrile, Methanol, Water.

Chromatographic conditions

Column (4.6×150mm)5µ	:	Agilent C18 column
Mobile phase ratio	:	Methanol: ACN (70: 30 % v/v)
Detection wavelength	:	254 nm
Flow rate	:	1.0ml/min
Injection volume	:	10µl
Column temperature	:	Ambient
Auto sampler temperature	:	Ambient
Run time	:	10min
Retention time	:	2.335 & 3.400 mins

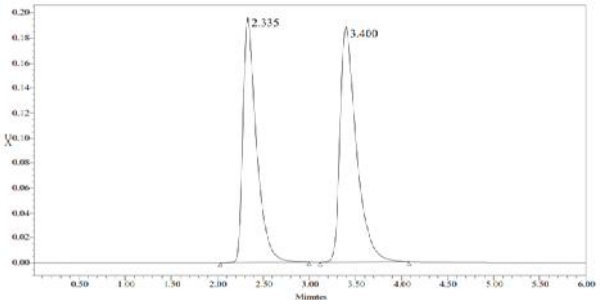

Fig 3: Optimized Chromatogram for Lamivudine and Zidovudine

Preparation of the Lamivudine and Zidovudine standard and sample solution

Sample solution preparation:

An equivalent tablet power such that 10 mg of Lamivudine and 20 mg Zidovudine tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent

Standard solution preparation:

10 mg Lamivudine and 20 mg Zidovudine working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Method Validation

Specificity:

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

Linearity:

10 mg of Lamivudine and 20 mg of Zidovudine working standard were accurately weighed and were transferred into a 10ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

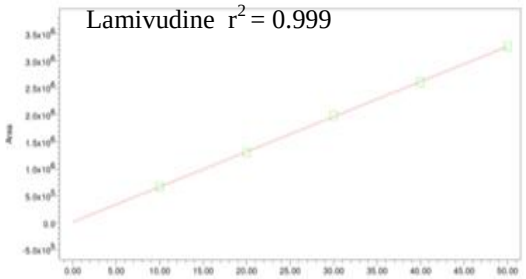

Fig 4: Calibration graph for Lamivudine

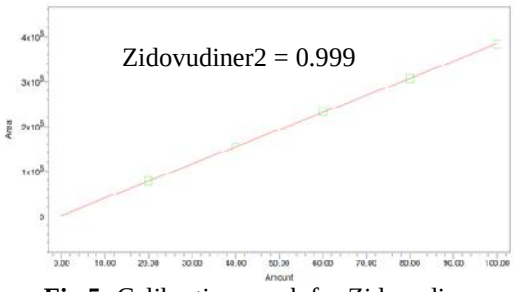

Fig 5: Calibration graph for Zidovudine

Range:

Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 10µg/ml-50µg/ml and 20µg/ml-100µg/ml of Lamivudine and Zidovudine respectively.

Accuracy:

10mg of Lamivudine and 20mg of Zidovudine working standard were accurately weighed and transferred into a

10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Precision:

Repeatability:

Preparation of stock solution: 10 mg of Lamivudine and 20 mg of Zidovudine working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Intermediate Precision/Ruggedness: To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on different days by using different make column of same dimensions.

Limit of detection (LOD):

LOD's can be calculated based on the standard deviation of the response (SD)and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Limit of quantification:

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Robustness:

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

System suitability:

10 mg of Lamivudine and 20 mg of Zidovudine working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

3. Results and discussion

Table 1: Assay results

S.No	Name of compound	Amount taken	%purity
1	Lamivudine	754.7	99.85
2	Zidovudine	735.6	100.34

Table 2: Linearity Results for Lamivudine

SI.No	Leniarity level	Concentration	Area
1	I	20 ppm	784928
2	II	40ppm	1524159
3	III	60ppm	2329360
4	IV	80ppm	3065982
5	V	100ppm	3830623
Correlation Coefficient			0.999

Table 3: Linearity Results for Zidovudine

SI.No	Leniarity level	Concentration	Area
1	I	20 ppm	784928
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4	IV	80ppm	3065982
5	V	100ppm	3830623
Correlation Coefficient			0.999

Table 4:50% Accuracy results for Lamivudine and Zidovudine

S.No	Lamivudine		Zidovudine	
	RT	Area	RT	Area
1	2.339	3062087	3.412	3574705
2	2.338	3065063	3.412	3574166
3	2.338	3070431	3.409	3583101
Mean		3065860		3577324
Std.Dev		4228.7		5009.9
%RSD		0.14		0.14

Table 5: 100% Accuracy results for Lamivudine and Zidovudine

S.No	Lamivudine		Zidovudine	
	RT	Area	RT	Area
1	2.338	3891186	3.415	4548377

2	2.338	3907240	3.422	4558118
3	2.337	3903606	3.421	4547232
Mean		3900678		4551243
Std.Dev		8418.1		5982.0
%RSD		0.22		0.13

Table 6:150% Accuracy results for Lamivudine and Zidovudine

S.No	Lamivudine		Zidovudine	
	RT	Area	RT	Area
1	2.337	4868463	3.420	5670786
2	2.340	4871561	3.4229	5674632
3	2.339	4883949	3.424	5682709
Mean		4874658		5676042
Std.Dev		8194.6		6085.3
%RSD		0.17		0.11

Table 7: Accuracy results for Lamivudine

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	3065860	5	4.96	99.91%	99.56%
100%	3900678	10	9.98	99.18%	
150%	4874658	15	15.02	99.60%	

Table 8: Accuracy results for Zidovudine

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	3577324	0.5	0.99	99.53%	99.47%
100%	4551234	1.0	1.05	99.38%	
150%	5676042	1.5	1.495	99.52%	

Table 9:% RSD results for Lamivudine and Zidovudine

S.No	Lamivudine		Zidovudine	
	RT	Area	RT	Area
1	2.335	1963566	3.408	2304558
2	2.332	1964716	3.406	2299453
3	2.333	1965030	3.409	2296908
4	2.330	1960856	3.404	2295001
5	2.331	1966445	3.407	2299613
Mean		1964123		2299107
Std.Dev		2094.9		3597.7
%RSD		0.11		0.16

Table 10: Intermediate precision results for Lamivudine and Zidovudine

S.No	Lamivudine		Zidovudine	
	RT	Area	RT	Area
1	2.332	1984822	3.413	2316744
2	2.331	1985152	3.409	2314478
3	2.330	1985353	3.408	2314400
4	2.332	1987338	3.412	2313639
5	2.330	2004113	3.408	2332909
Mean		1989356		2318434
Std.Dev		28308.1		8174.5
%RSD		0.42		0.35

Table 11: System suitability results for Lamivudine and Zidovudine

Drug	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing

Lamivudine	0.8	2934	1.6
	1	**2284**	**1.7**
	1.2	2911	1.7
Zidovudine	0.8	2427	1.6
	1	**2886**	**1.7**
	1.2	2336	1.7

Table 12: System suitability results for Lamivudine and Zidovudine

Drug	Change in organic composition in the mobile phase	**System suitability results**	
		USP Plate Count	**USP Tailing**
Lamivudine	5 % less	2866.1	1.7
	***Actual**	**2885**	**1.7**
	5 % more	2711.2	1.7
Zidovudine	5 % less	2457	1.7
	***Actual**	**2451**	**1.6**
	5 % more	2336.1	1.7

4. Conclusion

A new method was established for simultaneous estimation of Lamivudine and Zidovudine by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Lamivudine and Zidovudine by using Agilent C18 column (4.6×150mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol:Acetonitrile, detection wavelength was 254 nm. The % purity of Lamivudine and Zidovudine was found to be 99.85 % and 100.34% respectively. The system suitability parameters for Lamivudine and Zidovudine such as theoretical plates and tailing factor were found to be 2284, 1.7 and 2886 and 1.7, the resolution was found to be 5.4. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Lamivudine and Zidovudine was found in concentration range of 10µg-50µg and 20µg-100µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.10 and 0.1, % RSD for intermediate precision was 0.4 and 0.3 respectively. The precision study was precision, robustness and repeatabilty.LOD value was 2.17 and 0.0372 and LOQ value was 6.60 and 0.1125 respectively.

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