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R E S E A E 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 99.74% and 100.17% 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 (r^2) 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

A R T I C L E I N F O

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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 antiretrovirals such as zidovudine and abacavir. Lamivudine may be included as part of post-exposure prevention in those who have been potentially exposed to HIV. Lamivudine is taken by mouth as a liquid or tablet. Common side effects include nausea, diarrhea, headaches, feeling tired, and cough. Serious side effects include liver disease, lactic acidosis, and worsening hepatitis B among those already infected. It is safe for people over three months of age and can be used during pregnancy. The medication can be taken with or without food. Lamivudine is a nucleoside reverse transcriptase inhibitor and works by blocking the HIV reverse transcriptase and hepatitis B virus polymerase.

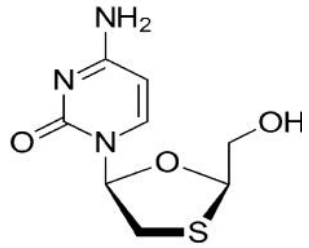


Fig 1: Structure of Lamivudine

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. It may be used to prevent mother-to-child spread during birth or after a needlestick injury or other potential exposure.It is sold both by itself and together as lamivudine/zidovudine and abacavir/lamivudine/zidovudine. It can be used by mouth or by slow injection into a vein.Common side effects include headaches, fever, and nausea. Serious side effects include liver problems, muscle damage, and high blood lactate levels. It is commonly used in pregnancy and appears to be safe for the baby. ZDV is of the nucleoside analog reverse-transcriptase inhibitor (NRTI) class. It works by inhibiting the enzyme reverse transcriptase that HIV uses to make DNA and therefore decreases replication of the virus.

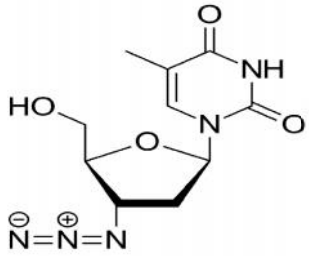


Fig 2: Structure of Zidovudine

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.

Chemicals

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

Chromatographic Conditions:

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

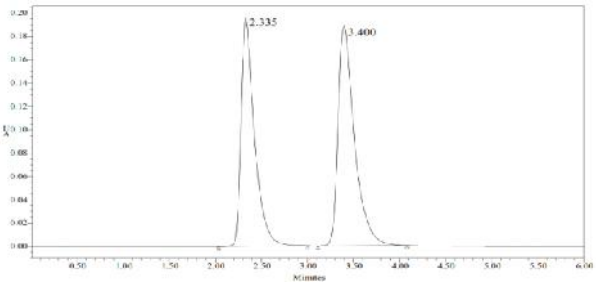


Fig 3: Optimized Chromatogram

Observation: The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks, so it is taken as final method.

Preparation of the individual Lamivudine standard preparation:

10 mg of Lamivudine working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 0.2 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent. Final concentration is 20µg/ml.

Preparation of the individual Zidovudine standard preparation:

10 mg of Zidovudine working standard was accurately weighed and transferred into a 10 ml 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 (Stock solution).Further pipette out 0.4 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent. Final concentration is 40 µg/ml. 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(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

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 (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

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(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

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 (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Procedure: 10µL of the blank, standard and sample were injected into the chromatographic system and areas for the Lamivudine and Zidovudine the peaks were used for calculating the % assay by using the formulae.

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.

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):

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 Discussions

Linearity:

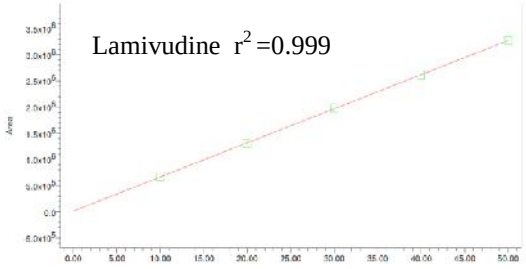


Fig 4: Showing calibration graph for Lamivudine

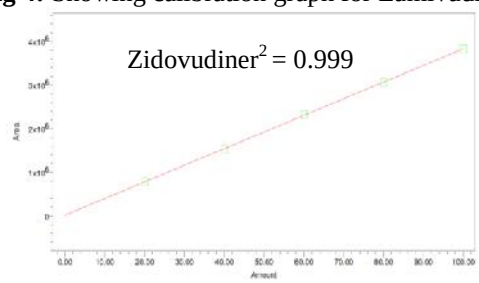
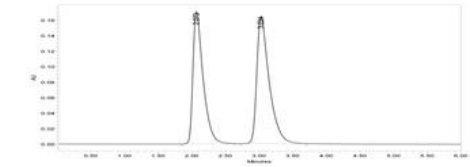


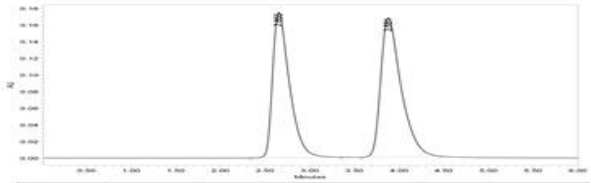
Fig 5: Showing calibration graph for Zidovudine

Robustness:



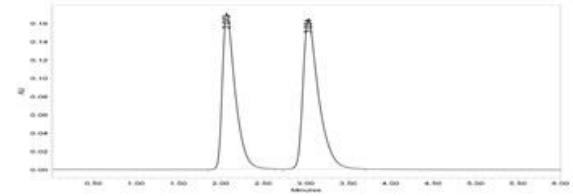
	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Resolution	USP Tailing	USP Plate Count
1	Lamiv	2.073	1794423	171911		1.7	2911.2
2	Zidov	3.034	2098583	164711	3.03	1.7	2336.7

Fig 6: Chromatogram showing more flow rate 1.2ml/min



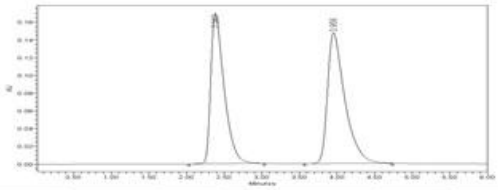
	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Resolution	USP Tailing	USP Plate Count
1	Lamivudine	2.655	2292934	175397		1.6	2934.8
2	Zidovudine	3.881	2640647	167695	3.12	1.6	2427.8

Fig 7: Chromatogram showing less flow rate 0.8 ml/min



	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Resolution	USP Tailing	USP Plate Count
1	Lamivudine	2.073	1794423	171911		1.7	2711.2
2	Zidovudine	3.034	2098583	164711	3.03	1.7	2336.7

Fig 8: Chromatogram showing more organic phase ratio



	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Resolution	USP Tailing	USP Plate Count
1	Lamivudine	2.387	2094744	170021		1.7	2866.0
2	Zidovudine	3.956	2366505	148168	4.08	1.7	2452.7

Fig 9: Chromatogram showing less organic phase ratio

Table No 1: 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 No 2: Linearity Results for Zidovudine

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 No 3: Details of 50%, 100% and 150% Accuracy

Accuracy 50%

Name: Lamivudine

	Name	RT	Area
1	Lamivudine	2.339	3062087
2	Lamivudine	2.338	3065063
3	Lamivudine	2.338	3070431
Mean			3065860
Std. Dev.			4228.7
% RSD			0.14

Name: Zidovudine

	Name	RT	Area
1	Zidovudine	3.412	3574705
2	Zidovudine	3.412	3574166
3	Zidovudine	3.409	3583101
Mean			3577324
Std. Dev.			5009.9
% RSD			0.14

Accuracy -100%

Name: Lamivudine

	Name	RT	Area
1	Lamivudine	2.338	3891186
2	Lamivudine	2.338	3907240
3	Lamivudine	2.337	3903606
Mean			3900678
Std. Dev.			8418.1
% RSD			0.22

Name: Zidovudine

	Name	RT	Area
1	Zidovudine	3.415	4548377
2	Zidovudine	3.422	4558118
3	Zidovudine	3.421	4547232
Mean			4551243
Std. Dev.			5982.0
% RSD			0.13

Accuracy 150%

Name: Lamivudine

	Name	RT	Area
1	Lamivudine	2.337	4868463
2	Lamivudine	2.340	4871561
3	Lamivudine	2.339	4883949
Mean			4874658
Std. Dev.			8194.6
% RSD			0.17

Name: Zidovudine

	Name	RT	Area
1	Zidovudine	3.420	5670786
2	Zidovudine	3.429	5674632
3	Zidovudine	3.424	5682709
Mean			5676042
Std. Dev.			6085.3
% RSD			0.11

Table No 4: Showing 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 No 5: Showing 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 No 6: Showing% RSD results for Lamivudine and Zidovudine

Name: Lamivudine

	Name	RT	Area
1	Lamivudine	2.335	1963566
2	Lamivudine	2.332	1964716
3	Lamivudine	2.333	1965030
4	Lamivudine	2.330	1960856
5	Lamivudine	2.331	1966445
Mean			1964123
Std. Dev.			2094.9
% RSD			0.11

Name: Zidovudine

	Name	RT	Area
1	Zidovudine	3.408	2304558
2	Zidovudine	3.406	2299453
3	Zidovudine	3.409	2296908
4	Zidovudine	3.404	2295001
5	Zidovudine	3.407	2299613
Mean			2299107
Std. Dev.			3597.7
% RSD			0.16

Table No 7: Showing results for intermediate precision of Lamivudine and Zidovudine

Name: Lamivudine

	Name	RT	Area
1	Lamivudine	2.332	1984822
2	Lamivudine	2.331	1985152
3	Lamivudine	2.330	1985353
4	Lamivudine	2.332	1987338
5	Lamivudine	2.330	2004113
Mean			1989356
Std. Dev.			8308.1
% RSD			0.42

Name: Zidovudine

	Name	RT	Area
1	Zidovudine	3.413	2316744
2	Zidovudine	3.409	2314478
3	Zidovudine	3.408	2314400
4	Zidovudine	3.412	2313639
5	Zidovudine	3.408	2332909
Mean			2318434
Std. Dev.			8174.5
% RSD			0.35

Table No 8: Showing system suitability results for Lamivudine

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	2934	1.6
2	1	2284	1.7
3	1.2	2911	1.7

Table No 9: Showing system suitability results for Zidovudine

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	2427	1.6
2	1	2886	1.7
3	1.2	2336	1.7

Table No 10: Showing system suitability results for Lamivudine

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	2866.1	1.7
2	*Actual	2885	1.7
3	5 % more	2711.2	1.7

Table No 11: Showing system suitability results for Zidovudine

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	2457	1.7
2	*Actual	2451	1.6
3	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 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 99.74% and 100.17% 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 (r^2) 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.Hence the suggested RP-HPLC method can be used for routine analysis of Lamivudine and Zidovudine in API and Pharmaceutical dosage form.

5. References

[1] Shaik Karishma , Shankar Subramaniam, Meenakshi Sundaram Muthuraman and Arvind Sivasubramanian et al, RP-HPLC Analytical Method Development And Validation For Lamivudine And Zidovudine In Pharmaceutical Dosage Forms. International Journal of PharmTech Research, Vol.5, No.3, pp 1321-1331, July-Sept 2013

[2] Palani Venkatesh,Mahesh Daggumati, Development and validation of a normal-phase HPTLC method for the simultaneous analysis of Lamivudine and Zidovudine in fixed-dose combination tablets. Journal of Pharmaceutical Analysis,Volume 2, Issue 2, April 2012, Pages 152–155.

[3] T. Raja and A. Lakshmana Rao et al, Development and Validation of RP-HPLC Method for the Estimation of Abacavir, Lamivudine and Zidovudine in Pharmaceutical Dosage Form. International Journal of PharmTech Research. Vol. 3, No.2, pp 852-857, April-June 2011

[4] Manikanta Kumar A, B. Naga,Sandhya, Mahesh Nasare, V. V. L. N Prasad, Prakash V Diwan et al, Development and Validation of RP-HPLC method for simultaneous estimation of Lamivudine and Efavirenz in the Pharmaceutical Dosage Form. Journal of Advanced Pharmacy Education & Research Oct-Dec 2012 Vol 2 Issue 4.

[5] Yadavalli Rekha , Yellina Haribabu , Sheeja Velayudhankutty , Sosamma Cicy Eapen and Jane Mary, Method Development and Validation for the Simultaneous Estimation of Efavirenz, Lamivudine and Zidovudine through Stability indicating RPHPLC Method. Research Journal of Pharmaceutical Sciences, Vol. 2(4), 10-18, May (2013).

[6] Sindhura D , A Kishore Agarwal, Analytical Method Development And Validation For The Simultaneous Estimation Of Lamivudine, Zidovudine And Efavirenz By Rp-Hplc In Bulk And Pharmaceutical Dosage Forms.

[7] Mirna El Barkil et al, Method Development And Validation of A RP-HPLC Method for The Simultaneous Estimation of Lamivudine and Zidovudine Combination In Tablet Formulations.

[8] S.Jayaseelan, S.Suresh, G.Sathishkumar, V.Sekar, P.Perumal. Bioanalytical Method Development And Validation Of Lamivudine By Rp-Hplc Method. International Journal of ChemTech Research, Vol.2, No.1, pp 163-167, Jan-Mar 2010.

[9] Dhara S. Bhavsar, Bhavini N. Patel, and Chhaganbhai N. Patel, RP-HPLC method for simultaneous estimation of tenofovir disoproxil fumarate, lamivudine, and efavirenz in combined tablet dosage form. Pharm Methods. 2012 Jul-Dec; 3(2): 73–78.

[10] 29. D.K.Mandloi, P.K.Tyagi , V.K.Rai , S. Dey , R.K Ashada , P.Mohanraj , Method development and validation of RP- HPLC in the application of invitro dissolution study of Lamivudine in bulk drug and tablet formulation. Journal of Chemical and Pharmaceutical Research, 2009, 1(1): 286-296.