Research Article

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DEVELOPMENT AND VALIDATION OF AN ASSAY METHOD FOR LAMIVUDINE AND ABACAVIR COMBINED TABLET FORMULATION BY RP-HPLC

*Narasimha Rao V Bathula, Gayathri Devi Ketineni

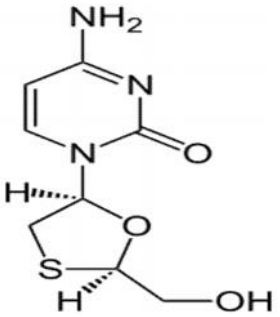
Victoria College of Pharmacy, Challavaripalem (village), Nallapadu (via), Guntur (District), Andhra Pradesh, India - 522 005.

Abstract

The aim of the present analytical research is to develop a simple, precise, accurate, rapid and economic RP-HPLC method for the assay of Lamivudine and Abacavir in combined tablet formulation. Till to date no accurate and precise RP-HPLC method is developed for the combined estimation of Lamivudine and Abacavir in combined tablet formulation. The main objective of this study is to validate the developed method by using parameters Specificity, Linearity, Precision, and Accuracy.

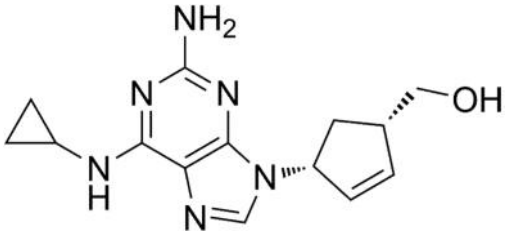
Keywords: Abacavir, Lamivudine, RP-HPLC.

Introduction



Lamivudine

Chemically lamivudine is 4-amino-1-((2R, 5S)-2-(hydroxyl methyl)-1,3-oxathiolan-5-yl) pyrimidin-2(1H)-one. Lamivudine is a synthetic nucleoside analogue with potent activity against HIV virus (Type-I) and hepatitis-B.



Abacavir

Chemically abacavir sulphate is [(1S,4R)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9yl] cyclo pent-2-en-1-yl] methanol sulphate. Abacavir is a carboxylic synthetic nucleoside analogue with activity against HIV virus (type-I). These both drugs act by inhibiting reverse transcriptase enzyme[1-4]. In the recent past Lamivudine and Abacavir combined formulations are designed as they exhibit synergistic effect in activity against

Author for Correspondence:

Narasimha Rao V Bathula,
C/o K. Anjaneyulu, Door No: 16-295,
Opp: Jaya nursing home, Kalamandhir center,
Chilakaluri pet, Guntur (dist), Andhra pradesh, India.
E-mail: gayathridevi.ketineni4@gmail.com

HIV-virus. For the analysis of Lamivudine and Abacavir individually UV, RP-HPLC and HPTLC methods are reported. Analytical methods such as UV and HPTLC are available for the combined estimation of Lamivudine and Abacavir[5-6].

Materials and method

Potassium dihydrogen ortho phosphate, triethylamine, methanol, acetonitrile, water, lamivudine WRS (99.8%), abacavir sulphate WRS (99.7%), ABEC-L (labell claim 300 mg of lamivudine and 600mg of abacavir). HPLC empower software, aliance 2695, detector 2487 model. UV spectrophotometer UV win 5 software, UV-3000+ model.

Chromatographic Conditions

Column	: Hypersil BDS C18 (100 X 4.6 mm) 5µ
Pump mode	: Isocratic
Flow rate	: 0.6 mL/min
Detection	: UV, 278 nm
Injection volume	: 20 µL
Column oven temperature	: Ambient
Run time	: 9 minutes

Standard Stock Solution

Accurately 50.04 mg of Lamivudine and 117.21 mg of Abacavir sulphate (equivalent to 100.0 mg of Abacavir) working standards were weighed and transferred into a 50 mL clean dry volumetric flask and about 10 mL of diluent was added, sonicated for 10min to dissolve completely and volume was made up to the mark with the diluent and filtered through 0.45 µ Millipore Nylon filter.

Standard Solution

4 mL of standard stock solution was pipetted into a 100 mL volumetric flask and diluted up to the mark with diluent.

Sample Stock Solution

20 tablets were weighed and average weight of tablet was determined. The tablets were crushed into a fine powder. Accurately weighed and transferred 232.12 gm of powder equivalent to 50 mg of Lamivudine into a 50 mL clean dry volumetric flask added about 10 mL of diluent and sonicated for 20 minutes. Volume was made up to the mark with the diluent and centrifuged at 5000 RPM for 10 minutes.

Sample Solution

4 mL of supernatant sample stock solution solution was pipetted into a 100 mL volumetric flask and diluted up to the mark with diluent and filtered through 0.45 µ Millipore Nylon filter.

Chromatographic Procedure of Assay

System Suitability

20 µL of the standard solution was injected into the chromatographic system and chromatogram was recorded.

Assay

20 µL of the standard solution was injected five times into the chromatographic system, chromatograms were recorded and peak areas were measured.

20 µL of the sample solution was injected in duplicate into the chromatographic system, chromatograms were recorded and peak areas were measured.

Acceptance Criteria

1. RSD for the peak areas of responses of five replicate injections of the standard solution is not more than 2.0%.
2. The number of theoretical plates (N) for the Lamivudine and Abacavir peaks is NLT 2000.
3. The Tailing factor (T) for the Lamivudine and Abacavir peaks is NMT 2.0

Calculation for Lamivudine

$$\text{Amount present} = \frac{A_{T1}}{A_{S1}} \times \frac{D_{S1}}{D_{T1}} \times \frac{P_1}{100} \times AW$$

Where,

A_{T1} = Average area counts of Lamivudine peak in chromatogram of sample solution

A_{S1} = Average area counts of Lamivudine peak in chromatogram of standard solution

D_{S1} = Dilution factor for the standard solution

D_{T1} = Dilution factor for the sample solution

P_1 = Percentage potency of Lamivudine working standard used (as is basis)

AW = Average weight of tablet

$$\% \text{ Labeled Amount} = \frac{\text{Amount of Lamivudine}}{\text{Label claim of Lamivudine}} \times 100$$

Calculation for Abacavir

$$\text{Amount present} = \frac{A_{T2}}{A_{S2}} \times \frac{D_{S2}}{D_{T2}} \times \frac{P_2}{100} \times M.F \times AW$$

Where,

A_{T2} = Average area counts of Abacavir peak in chromatogram of sample solution.

A_{S2} = Average area counts of Abacavir peak in chromatogram of standard solution

D_{S2} = Dilution factor for the standard solution

D_{T2} = Dilution factor for the sample solution

P_2 = Percentage potency of Abacavir working standard used (as is basis)

M.F = Molecular factor

AW = Average weight of tablet

$$\% \text{ Labeled Amount} = \frac{\text{Amount of Abacavir}}{\text{Label claim of Abacavir}} \times 100$$

Method of Validation

Specificity

The retention times obtained from working standard and test samples were compared for identification.

Linearity

A series of solutions of drug substance standard were prepared in the concentration range from 50% to 300% of test concentration to demonstrate linearity for assay by using single plot and injected in to the chromatographic system. A calibration graph is plotted between amount of drug (µg/mL) and chromatographic peak area (mV).

Precision

System Precision

The system precision was established by injecting six replicate injections of standard solution in to the chromatographic system by maintaining the optimized chromatographic conditions.

Method Precision

Six assay samples of drug product at 100% of the working sample concentration were prepared and injected into the chromatographic system (Set-I).

Accuracy

Sample solutions prepared separately by addition of standard stock at 50%, 100% and 150% of working sample concentration were injected three times into the chromatographic system.

Results

Table No. 01: Data of system suitability

S.no	Name	Retention time(min)	Area (µV*sec)	Height (µV)	USP Resolution	USP plate count	USP Tailing	accuracy (recovery values)
1	lamivudine	2.128	3628811	242040.4	3.01	3439	1.5	99.4%-100.2%
2	Abacavir sulphate	3.214	6910890	389805.7		3787	1.6	99.8%-100.4%

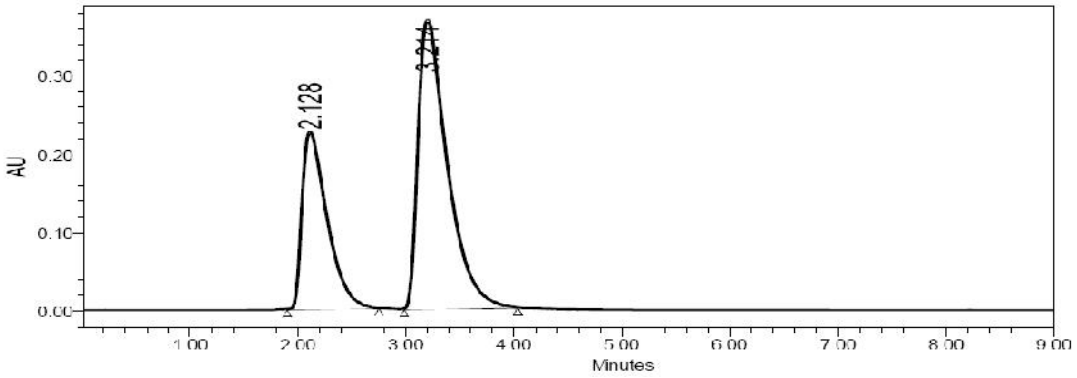


Fig. No. 01: Chromatogram of system suitability

Table No. 02: Data of standard chromatograms

Injection	Retention Time		Peak Area	
	Lamivudine	Abacavir sulphate	Lamivudine	Abacavir sulphate
1	2.130	3.216	3612114	6906784
2	2.124	3.208	3613316	6910847
3	2.132	3.216	3612124	6907887
4	2.128	3.214	3620116	6876906
5	2.126	3.215	3621411	6912548
Mean			3615816	6902994
SD			4565.6	14763.4
% RSD			0.12	0.21

Chromtograms of standard

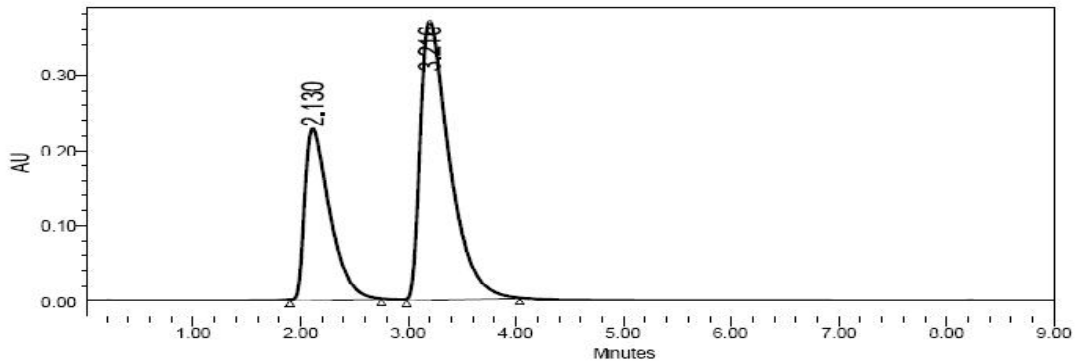


Fig. No. 02: Chromatogram No: 1

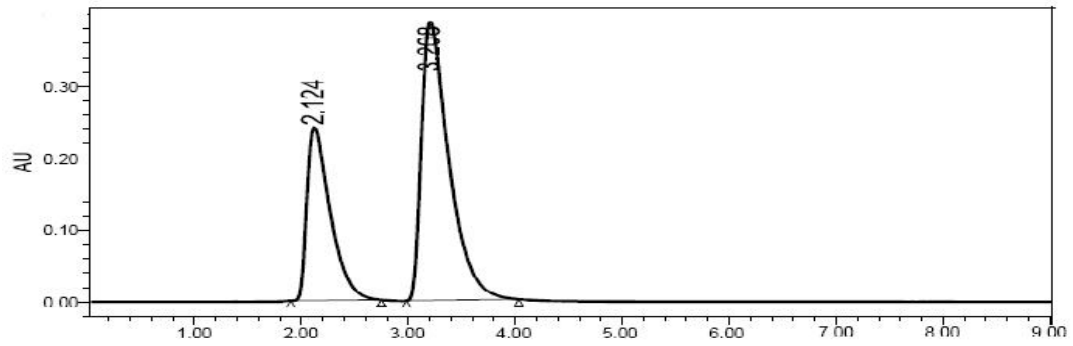


Fig. No. 03: Chromatogram No: 2

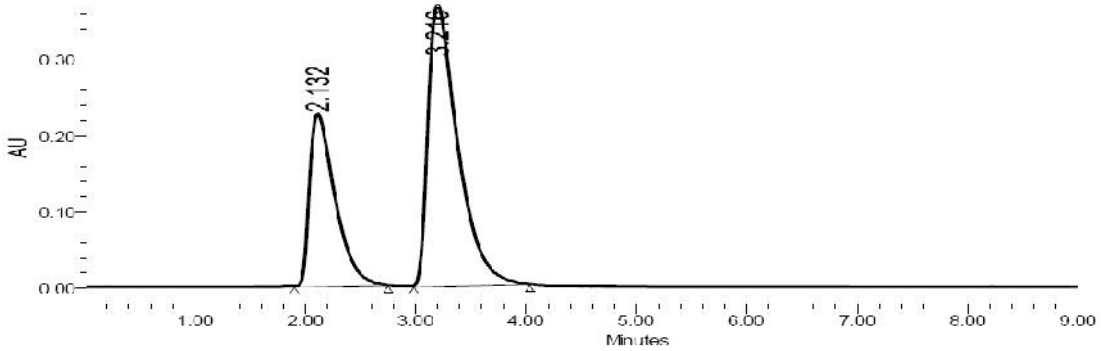


Fig. No. 04: Chromatogram No: 3

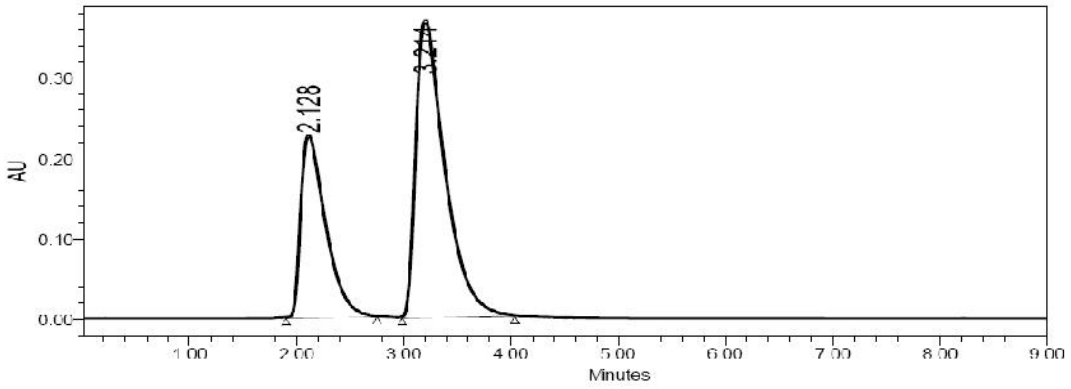


Fig. No. 05: Chromatogram No: 4

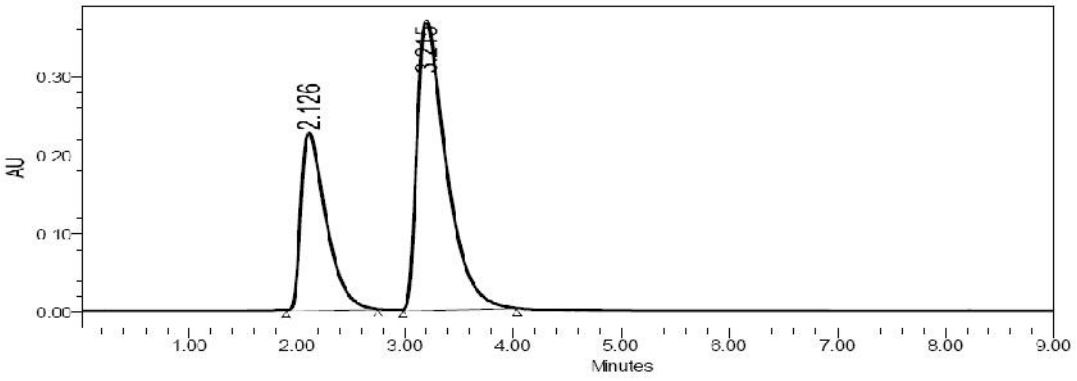


Fig. No. 06: Chromatogram No: 5

Sample chromatograms

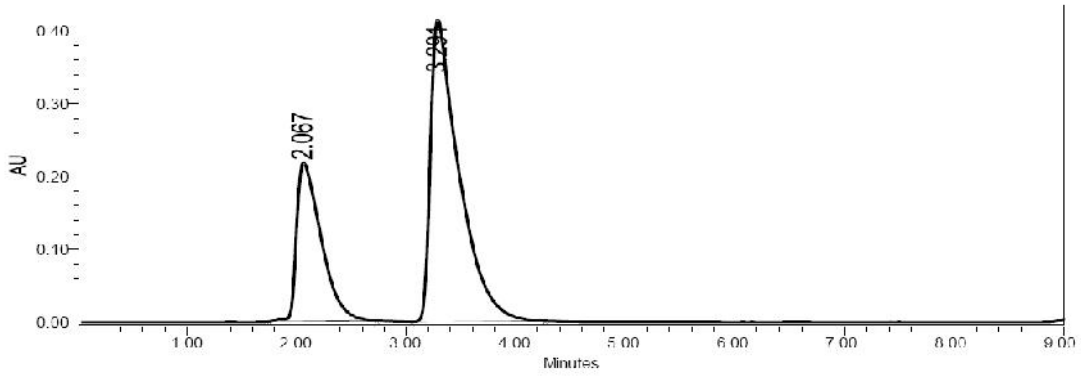


Fig. No. 07: Chromatogram No: 1

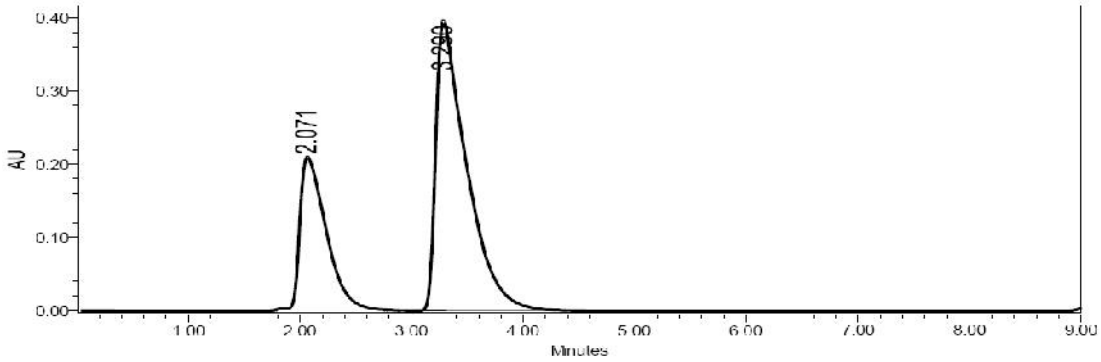


Fig. No. 08: Chromatogram No: 2

Table No. 03: Data of sample chromatograms

Injection	Retention Time		Peak Area	
	Lamivudine	Abacavir sulphate	Lamivudine	Abacavir sulphate
1	2.067	3.291	3581677	6883814
2	2.071	3.290	3576624	6878314
Mean			3579150	6881064
SD			3573	3889
% RSD			0.09	0.05

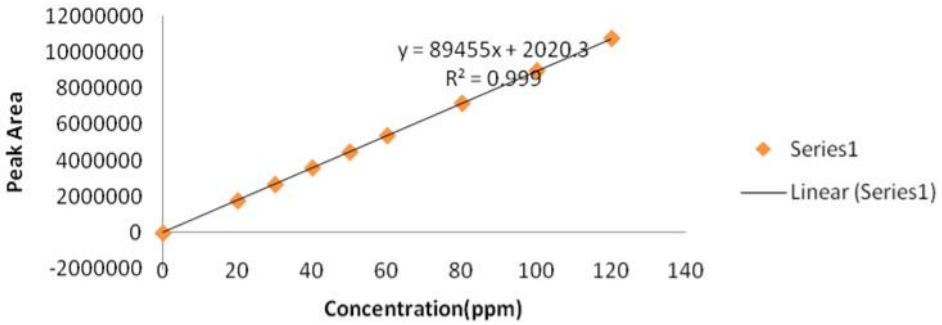


Fig. No. 09: Calibration curve of Lamivudine

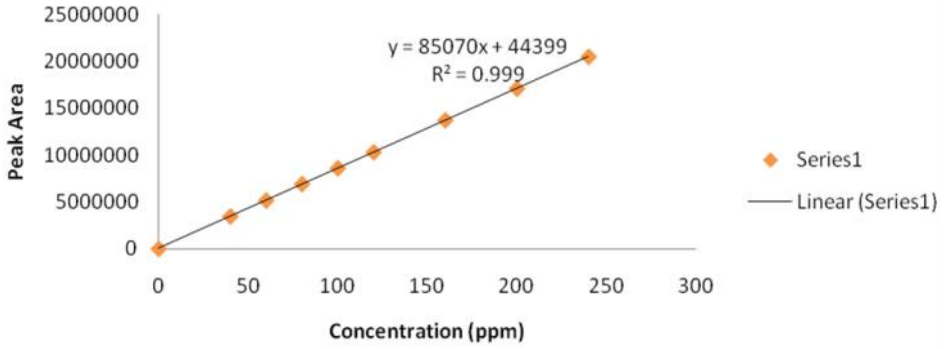


Fig. No. 10: Calibration curve of Abacavir

Table No. 04: Validation parameters

S.No	Parameter	Result	
		Lamivudine	Abacavir
1	Linearity	20-120 µg/mL Correlation coefficient = 0.999	40-240 µg/mL Correlation coefficient = 0.999
2	System precision	%RSD = 0.30	%RSD = 0.17
3	Method precision	%RSD = 0.21	%RSD = 0.21
4	Accuracy	Recovery values = 99.4%-100.2%	Recovery values = 99.8%-100.4%

Discussion

A simple, sensitive, rapid and economic RP-HPLC method was developed and validated for the assay of Lamivudine and Abacavir in combined tablet formulation. This method yielded high recoveries with good linearity and precision. It can be concluded that the proposed method is a good approach for obtaining reliable results and found to be suitable for the routine analysis of Lamivudine and Abacavir in combined tablet formulation.

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