CODEN (USA): IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.375937

Available online at: http://www.iajps.com *Research Article*

STABILITY-INDICATING METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF LAMIVUDINE, ABACAVIR, DOLUTEGRAVIR IN PHARMACEUTICAL DOSAGE FORMS

SK. Shaista Fatima, P. Nagaraju*, V. Mounika, G. Indira Priyadarshini and V. Vasu Naik

Professor, Department of Pharmaceutical Analysis,
Hindu College of Pharmacy, Amaravathi Road, Guntur – 522002,
Andhra Pradesh, India.

Received: 20 February 2016 **Accepted:** 26 February 2017 **Published:** 28 February 2017

Abstract:

A simultaneous stability-indicating reversed-phase high performance liquid chromatography (HPLC) method for analysis of lamivudine (LAMI), abacavir (ABA), dolutegravir (DOLU) in the bulk drug and in the formulation was developed. Compounds were separated on Inersil ODS (4.6 × 250 mm x 5 mm). A gradient program of mobile phase at different proportions of Phosphate Buffer pH-7 & Acetonitrile was used. The retention times of LAMI, ABA & DOLU were 2.555, 4.282, 7.101 minutes (mins.) respectively. The drugs were subjected to the stress conditions of acid, base, oxidative & thermal degradation. The degradation products were well resolved from main peak and its impurities, proving the stability-indicating ability of the method. The method was linear in the concentration range of 0– 150µg/mL, 0-300 µg/mL and 0-25 µg/mL for LAMI, ABA & DOLU respectively. The method was accurate and precise with a limit of detection and limit of quantitation of 0.31 and 0.94 µg/ mL, 0.21 and 0.65 µg/ mL and 0.48 and 1.46 µg/ mL for LAMI, ABA & DOLU respectively. The method was applied for the analysis of LAMI, ABA & DOLU in the presence of its degradation products and commonly used excipients and was found to be specific. The developed method is stability indicating, precise and specific which can be applied for the routine analysis.

Key words: *Lamivudine, Abacavir, Dolutegravi and stability-indicating*

Corresponding author:

P. Nagaraju,
Professor,
Department of Pharmaceutical Analysis,
Hindu College of Pharmacy,
Amaravathi Road, Guntur – 522002,
Andhra Pradesh, India.
Contact: ***09985304304***
E-mail: ***pappulanagaraju@gmail.com***

QR code

Please cite this article in press as -.Nagaraju et al, ***Stability-Indicating Method Development and Validation of RP-HPLC Method for Simultaneous Estimation of Lamivudine, Abacavir, Dolutegravir in Pharmaceutical Dosage Forms****, Indo Am. J. P. Sci, 2017; 4(02).*

INTRODUCTION [1-10]:

Lamivudine (LAMI) which is chemically 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one is an analogue of cytidine. It can inhibit both types (1 and 2) of HIV reverse transcriptase and also the reverse transcriptase of hepatitis B virus. It is phosphorylated to active metabolites that compete for incorporation into viral DNA. They inhibit the HIV reverse transcriptase enzyme competitively and act as a chain terminator of DNA synthesis. The lack of a 3'-OH group in the incorporated nucleoside analogue prevents the formation of the 5' to 3' phosphodiester linkage essential for DNA chain elongation, and therefore, the viral DNA growth is terminated.

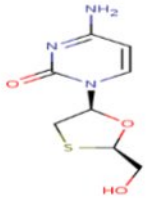

Fig 1a: Lamivudine

Abacavir, (ABA) which is chemically [(1S,4R)-4-[2-amino-6-(cyclopropylamino)-9H-pu rin-9-yl]cyclopent-2-en-1-yl]methanol, is a carbocyclic synthetic nucleoside analogue. Intracellularly, it is converted by cellular enzymes to the active metabolite carbovir triphosphate. Carbovir triphosphate is an analogue of deoxyguanosine-5`-triphosphate (dGTP). Carbovir triphosphate inhibits the activity of HIV-1 reverse transcriptase (RT) both by competing with the natural substrate d GTP and by its incorporation into viral DNA

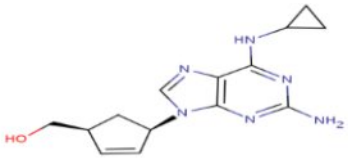

Fig 1b: Abacavir

Dolutegravi r(DOLA) which is chemically ((4R,12aS)-N-(2,4-difluorobenzyl)-7-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2H-pyrido [1',2':4,5] pyrazino[2,1-b] [1,3]oxazine-9-carboxamide, inhibits HIV integrase by binding to the integrase active site and blocking the strand transfer step of retroviral DNA integration which is essential for the HIV replication cycle.

There are several reported spectrophotometric and chromatographic methods in the literature for analysis of LAMI, ABA & DOLU individually but there is no reported method for the simultaneous stability indicating assay of the drugs in combination [11-34]. Hence LC method that was developed in the present work is advantageous because it enables stability indicating, accurate, specific and reproducible analysis of LAMI, ABA & DOLU

MATERIALS AND METHODS:

Instrumentation and Reagents:

Liquid chromatography was performed with a Waters 2695 series High performance liquid chromatography instrument equipped with Auto Sampler and DAD or UV detector. Empower 2 software was applied for data collection and processing. The separation was achieved on a Inertsil ODS (4.6 x 250mm, 5µm) analytical column.

LAMI, ABA & DOLU was obtained as a gift sample from Hetero Labs, Hyderabad, India.

Preperation of solutions:

Preparation of diluents: A mixture of Potassium Dihydrogen Orthophosphate of P^H-7and Acetonitrile was prepared and used as mobile phase.(**Gradient Mode)**

Standard stock solution: Accurately Weighed and transferred 30 mg of Lamivudine, 60 mg of Abacavir & 5 mg of Dolutegravir working Standards into individual 10 ml clean dry volumetric flask, diluent was added and sonicated for 10 minutes and make up to the final volume with diluent. From the above stock solution, 1 ml, 1ml, 1ml was pipetted out in to a 10 ml volumetric flask and then make up to the final volume with diluent. From above solution, 3ml, 3ml, 3ml was pipetted out into a 10 mL volumetric flask and then make up the final volume with diluent and thus we have (90 µg/ml Lamivudine, 180 µg/ml Abacavir & 15 µg/ml dolutegravir).

Sample stock solution:

20 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a three different 10 mL volumetric flask, 3/4th volume of diluent added and sonicated for 30 min, further the volume made up with diluent. From the above stock solution, 0.1 ml, 0.1ml, 0.1ml was pipetted out in to a 10 ml volumetric flask and then make up to the final volume with diluent. From above stock solution, 3ml, 3ml, 3ml was pipetted out into a 10 mL volumetric flask and then make up the final volume with diluent

Optimized Chromatographic Conditions:

Compounds were separated on a Inertsil ODS (4.6 x 250mm, 5µm) column with gradient program of Phosphate Buffer pH-7: Acetonitrile [Table 1] as mobile phase at a flow rate of 1.2 mL/min. Chromatography was performed at room temperature and the detection was carried out at 254 nm.

Table 1: Column with gradient program of Phosphate Buffer pH-7: Acetonitrile

TIME	FLOW ml/min	A(BUFFER pH-7.0)	B(ACETONITRILE)
0	1.2	75	25
5.0	1.2	30	70
8.5	1.2	75	25

Forced Degradation Studies:
Intentional degradation (n = 3) was attempted by using, heat, acid, base, and oxidizing agent. For acid degradation, 3 mL of working standard solution was refluxed with 2N hydrochloric acid (HCl) at 60°C for 1hour and then neutralized by adjusting pH to 7.0 with 0.1N sodium hydroxide (NaOH). For alkali degradation, 3 mL of working standard solution was refluxed with 0.1N NaOH at 60°C for 1hour and then neutralized by adjusting pH to 7.0 with 0.1N HCl. For oxidative degradation, 3 mL of working standard solution was refluxed with 3%w/v hydrogen peroxide (H_2O_2) by heating on water bath at 60°C for 1hour. For thermal degradation, 2mL of working standard solution was exposed to temperatures at 110°C for 24 hours. All these solutions except for were prepared in amber volumetric flasks. After completion of the degradation treatments the samples were cooled to room temperature, diluted with the diluent, and injected for chromatographic analysis.

Method Validation:
The method was validated in accordance with recognized guidelines.

System suitability:
The suitability of the system was studied by the values obtained for Theoretical plate, Resolution and tailing factor of the chromatogram of standard drugs and presented in the Table 3. The selectivity of the method was revealed by the repeated injection of mobile phase and no interference was found.

Linearity:
Six solutions containing LAMI, ABA & DOLU were prepared in diluents. Peak area and concentration data were treated by least squares linear regression analysis (n = 3).

Precision
Method precision was evaluated by injecting sample preparation at test concentration level of LAMI, ABA & DOLU for 6 times (n=3) on different HPLC system.
The system precision of test method was performed by injecting three portions from a standard solution on to the analytical column and the peak area data obtained then %RSD was calculated.

Accuracy:
Accuracy of the method confirmed by studying recovery at 3 different concentrations 50%, 100%, and 150% of these expected, in accordance with ICH guidelines, by replicate analysis (n=3). Standard drug solution was added to a pre analyzed sample solution and percentage drug content was measured.

LOD and LOQ:
LOD and LOQ were determined as the amounts for which the signal-to-noise ratios were 3:1 and 10:1, respectively.

Robustness:
Robustness of method was carried out with variation in pH, mobile phase composition and detection wavelength (± 2 nm).

RESULTS AND DISCUSSION:

The retention times of LAMI, ABA & DOLU under the chromatographic conditions described above were 2.555, 4.282, 7.101 mins respectively [Fig. 2.a]. Assay calculations are given in Table no. 2. System suitability data is given in table no. 3 where it is evaluated by theoretical plates and tailing factor. The peaks of the degradation products were well resolved from that of LAMI, ABA & DOLU [Fig. 2c–f]. There was no interference from the excipients commonly present in the formulation and from the mobile phase. It may therefore be inferred that no degradation of LAMI, ABA & DOLU in the pharmaceutical formulation was detected by using this method.

In validation of the assay, blank, yielded clean chromatograms [Fig.2.b]; with no interference from the excipients and mobile phase; this is indicative of the specificity of the method. The LOD and LOQ was 0.31, 0.94 and 0.21, 0.65 µg/ mL and 0.48, 1.46 µg/ mL, for LAMI, ABA & DOLU respectively. A plot of drug peak area against concentration [Fig. 3a 3b 3c] of LAMI, ABA & DOLU was linear over the concentration range 0–150µg/mL, 0 -300µg/ mL and 0- 25µg/ mL respectively. The regression equation was calculated by the least-square method for LAMI, Y = 22044X - 46592; correlation coefficient 0.999, for ABA, Y = 15639X+50671; correlation coefficient 0.999 and for DOLU Y = 32920X+50138; correlation coefficient 0.999. The method was found to be precise as the RSD <2 [Table 4].

The recovery studies were carried out by comparing method of three individual standards with each of three samples with same procedure from the formulation and injecting. The percentage recovery and percentage relative standard deviation of the percentage recovery were calculated and presented in Table 5. From the data obtained, added recoveries of standard drugs were found to be accurate. This quantitative recovery of the drugs indicates that there was no interference from excipients present in the formulation and the method is accurate whose results are shown in Table 5. LAMI, ABA & DOLU were found to be stable in the mobile phase for a period of 24hours, because no peaks corresponding to degradation products were observed and there was no significant change in the peak area of the drug (RSD <1%). The deliberate changes in the method have not much affected the peak tailing, theoretical plates and the percent assay. This indicates that the present method is robust (Table 6). Results of Degradation Studies are given in Table 7.

Chromatograms obtained from drugs and its degradation products:

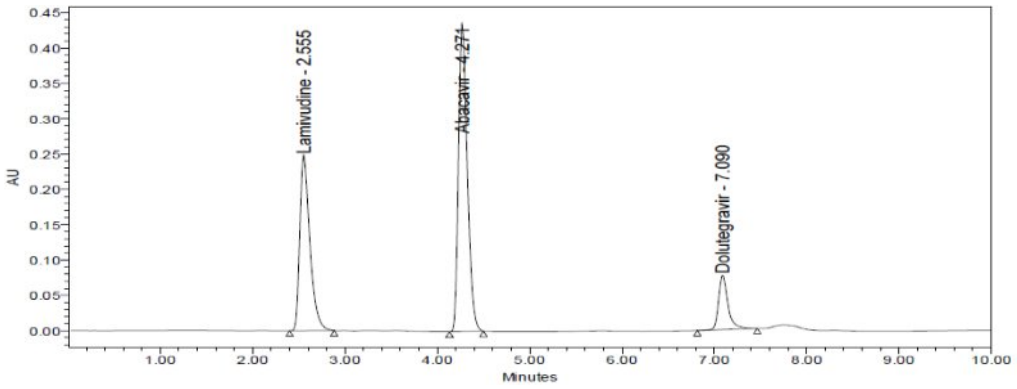

Fig. 2a: Chromatogram of Standard

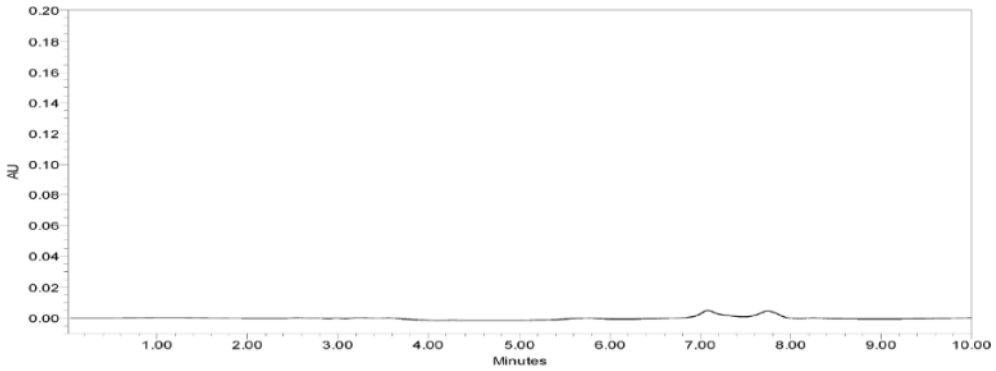

Fig. 2:b Chromatogram of Blank

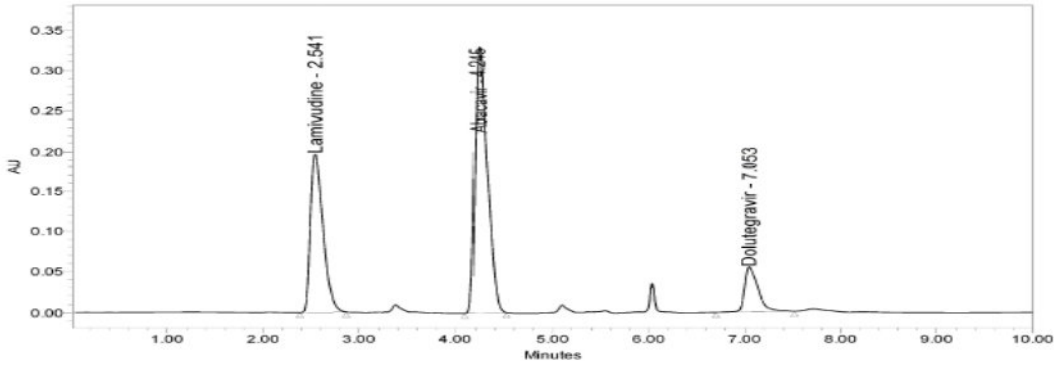

Fig. 2c: Acid Degradation

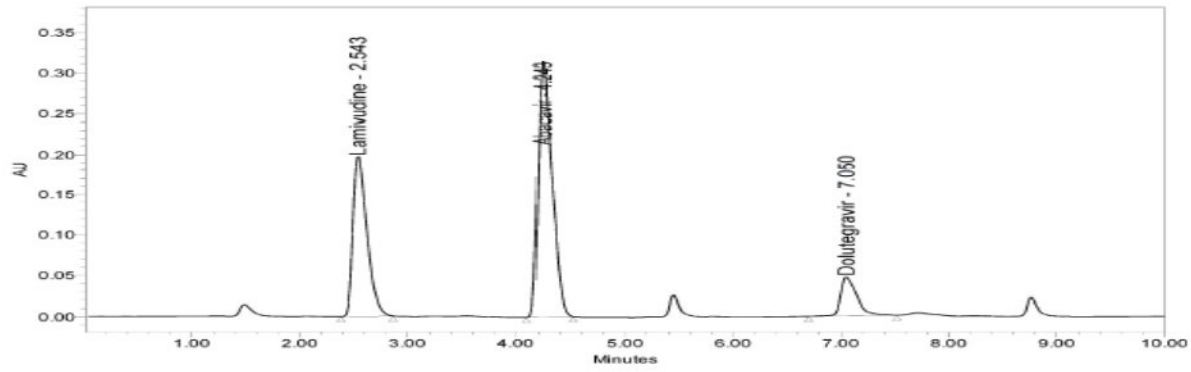

Fig. 2d: Alkali Degradation

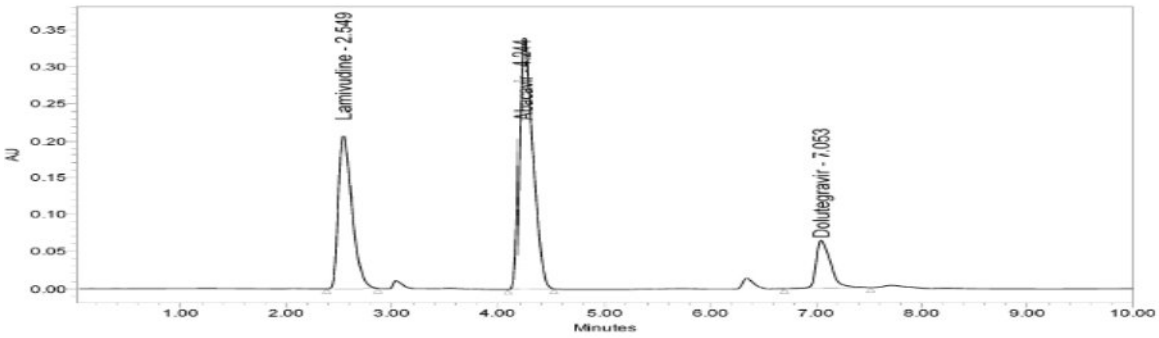

Fig. 2e: Thermal Degradation

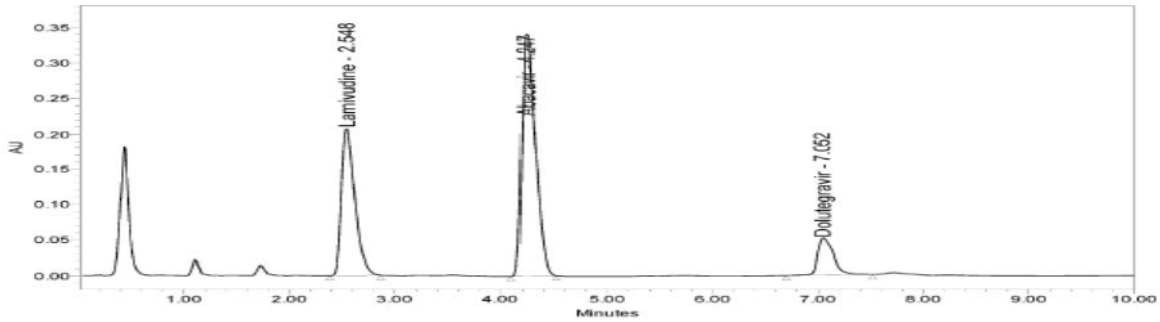

Fig.2f: Peroxide Degradation

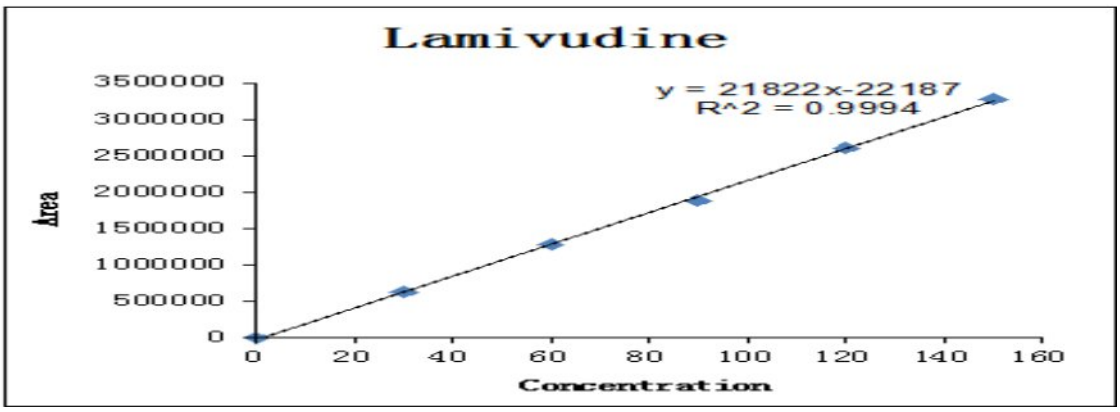

Fig.3a: Calibration Plot of Lami

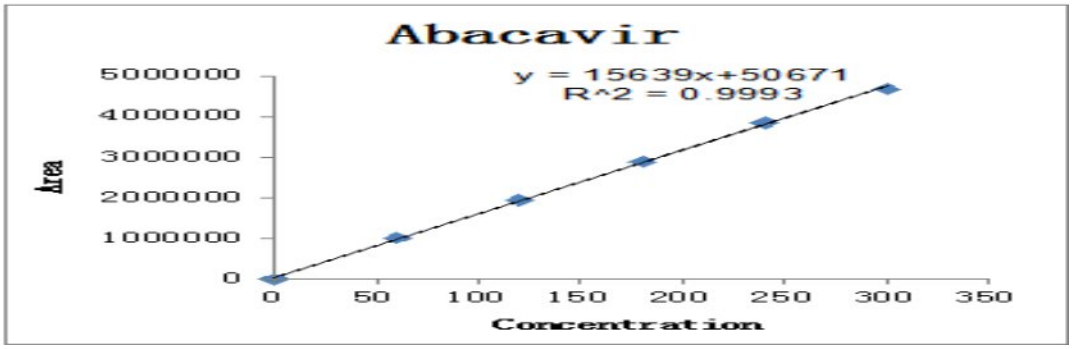

Fig.3b: Calibration Plot of Aba

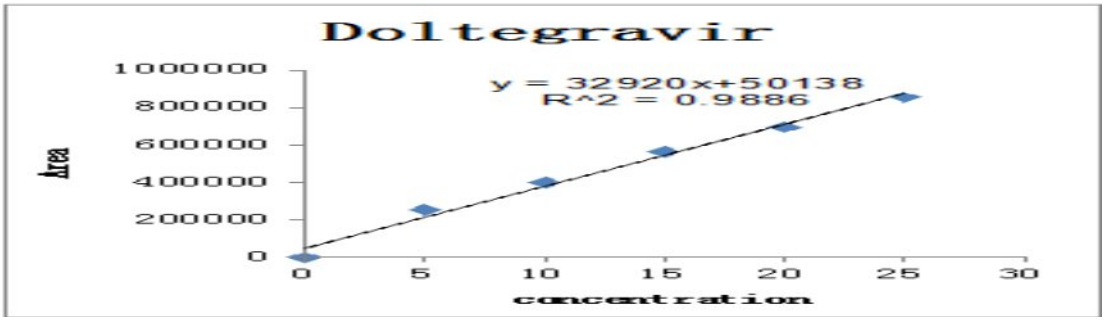

Fig.3c: Calibration Plot of Dolu

Table 2: Assay Result of LAMI, ABA & DOLU

S.No.	Drug	Label Claim	Amount Found*	%RSD
1.	Lamivudine	300 mg	299.65mg	99.88
2.	Abacavir	600 mg	591.84mg	98.64
3.	Dolutegravir	50 mg	49.1mg	98.0

* Mean of Three Determinations

Table 3: System Suitability

Parameters	Lamivudine	Abacavir	Dolutegravir	Acceptance Criteria
Tailing factor	1.48	1.31	1.21	NMT 2.0
No. of theoretical plates	2638	8195	22798	NLT 2000
Retention time (min)	2.555	4.282	7.101	-
Resolution	--	8.90	14.98	NLT 2.0

Table 4: Precision Data of the Proposed Method

	Lamivudine		Abacavir		Dolutegravir	
Injection	Method Precision	System Precision	Method Precision	System Precision	Method Precision	System Precision
1.	1923988	1880157	2964962	2930353	584343	572535
2.	1893524	1839839	2999786	2847284	582205	559339
3.	1899965	1876570	2927107	2901585	575084	570178
4.	1930246	1870271	2971382	2885735	584822	569599
5.	1889827	1837622	2908039	2922475	572613	579735
Mean	1914466	1860891.8	2960767	2987486.4	580850.3	570277.2
S.D	2368.562	2055.254	3640.389	3309.09827	5617.704	7325.668174
%RSD	1.23	1.10	1.22	1.14	0.96	1.28

Table 5: Accuracy Data (Triplicate Values at 50, 100 and 150 Percent Levels) of LAMI, ABA & DOLU

% Recovery of Lamivudine

Recovery Level (%)	Fixed concentration (µg/ml)	Spiked concentration (µg/ml)	Recovered concentration (µg/ml)	% Recovery
50	90	60	59.88	99.8
100	90	90	89.90	99.9
150	90	120	119.70	99.7

% Recovery of Abacavir

Recovery Level (%)	Fixed concentration (µg/ml)	Spiked concentration (µg/ml)	Recovered concentration(µg/ml)	% Recovery
50	180	150	149.8	99.6
100	180	180	178.9	99.3
150	180	210	209.03	99.5

% Recovery of Dolutegravir

Recovery Level (%)	Fixed concentration (µg/ml)	Spiked concentration (µg/ml)	Recovered concentration (µg/ml)	% Recovery
50	15	7.5	7.51	100.1
100	15	15	15.23	101.5
150	15	22.5	22.48	99.9

Table 6: Robustness Data of LAMI, ABA & DOLU

S.no	Robustness Condition	Peak Area of Lamivudine	Peak Area of Abacavir	Peak Area of Dolutegravir
1.	Flow rate 1.0 ml/min	2136130	3302422	692435
2.	Flow rate 1.4 ml/min	1784775	2766836	568517
3.	Wavelength 252nm	1855712	2791292	526489
4.	Wavelength 256nm	2001906	3111378	636050

Table 7: Forced Degradation Data

Stress condition	Lamivudine		Abacavir		Dolutegravir	
	% Assay	% Degradation	% Assay	% Degradation	%Assay	%degraded
Control	100	-	100	-	100	-
Acid	88.81	11.19	86.84	13.16	90.43	9.57
Alkaline	87.62	12.38	85.69	14.31	92.51	7.49
Peroxide	97.33	2.67	92.99	7.01	88.52	11.48
Thermal	93.74	6.26	93.74	6.36	98.27	1.73

CONCLUSION:

This RP-HPLC method for assay of lamivudine abacavir and dolutegravir is precise, specific, rapid, and stability-indicating. The method may be used to assess the stability of lamivudine abacavir and dolutegravir as the bulk drug and in its pharmaceutical formulation. Chromatographic analysis time of less than 20 min was advantageous for use of the method in routine analysis. It may be extended to study of lamivudine abacavir and dolutegravir and also analysis of the drug in plasma and other biological fluids.

REFERENCES:

1. http://www.drugbank.ca/drugs/DB01048
2. http://www.drugbank.ca/drugs/DB08930
3. http://www.drugbank.ca/drugs/DB00709
4. https://www.pharmgkb.org/pathway/PA166104634
5. https://www.pharmgkb.org/pathway/PA1658

60384
6. "WHO Model List of Essential Medicines" (PDF). World Health Organization. October 2013. Retrieved 22 April 2014.
7. "Statement on a Nonproprietary Name Adopted by the USAN Council" (PDF). American Medical Association (AMA). Retrieved 9 February 2014.
8. FDA approves new drug to treat HIV infection http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm364744.htm Aug. 12, 2013.
9. "U.S. FDA approves GlaxoSmithKline's HIV drug Tivicay". Reuters. 12 August 2013. Retrieved 13 February 2013.
10. "GSK wins priority status for new HIV drug in U.S". Reuters. 16 February 2013. Retrieved 18 February 2013.
11. T. Sudha et al RP-HPLC Method for the Simultaneous Estimation of Lamivudine and Abacavir Sulphate in Tablet Dosage Form International Journal on Pharmaceutical and Biomedical Research (IJPBR) Vol. 1(4), 2010, 108-113
12. Mohideen et al. Validated RP-HPLC method for Simultaneous Estimation of ABACVIR and LAMIVUDINE In Tablet dosage form International Journal of Pharmacy and Pharmaceutical Sciences , Vol 4, Suppl 1, 349-355
13. Chantelle Bennetto-Hood et al. A Sensitive HPLC-MS/MS Method for the Determination of Dolutegravir in Human Plasma Chromatogr B Analyt Technol Biomed Life Sci. Author manuscript; available in PMC 2015 Jan 15. Published in final edited form as: J Chromatogr B Analyt Technol Biomed Life Sci. 2014 Jan 15; 0: 225–232. Published online 2013 Dec 3. doi: 10.1016/j.jchromb.2013.11.054
14. C Rambabu et al Development and Validation of RP-UPLC Method for Simultaneous Estimation of Abacavir Sulphate and Lamivudine in Combined Tablet Dosage Form International Journal of Chem Tech Research CODEN(USA): IJCRGG ISSN : 0974-4290 Vol.4, No.3, pp 939-944, July-Sept 2012
15. D.K.Mandloi et al. Method development and validation of RP- HPLC in the application of in vitro dissolution study of Lamivudine in bulk drug and tablet formulation Journal of Chemical and Pharmaceutical Research, 2009, 1(1): 286-296
16. C.Palavan et al. A New RP- HPLC Method for the Simultaneous Estimation of Abacavir, Lamivudine and Zidovudine in Tablet Dosage Forms. AJPTR American journal of Pharm Tech Research ISSN NO 2249-3387, Accepted -18 January 2013
17. Alagar raja M. et al. Rp-Hplc Method Estimation And Its Validation Of Abacavir Sulfate In Tablet Formulation In Bulk And Pharmaceutical Formulations. International Journal of Biological & Pharmaceutical Research (IJBPR). 2012; 3(1): 1-5
18. J.V.L.N. SESHAGIRI RAO et al. Simultaneous Determination of Lamivudine, Zidovudine and Abacavir in Tablet Dosage Forms by RP HPLC Method ISSN: 0973-4945; CODEN ECJHAO E-Journal of Chemistry 2010, 7(1), 180-184
19. Honey Diana B et al. New validated rp-hplc method for simultaneous estimation of lamivudine and tenofovir disproxil fumarate in tablets International Journal of Advances in Pharmaceutical Analysis IJAPA Vol. 5 Issue 1 (2015) 10-13
20. S. Lavanya et al. RP-HPLC Method Development and Validation of Abacavir Sulphate in Bulk and Tablet Dosage Form International Journal of Pharma Sciences and Research (IJPSR) Vol 5 No 11 Nov 2014 pg.no: 790-798.
21. Vaishali P. Nagulwar et al. Development Of RP-HPLC Method For The Simultaneous Estimation Of ABACAVIR And LAMIVUDINE in Combined Tablet Dosage Form. International Journal of Pharmaceutical Applications ISSN 0976-2639. Vol 3, Issue 1, 2012, Pg 293-296.
22. V.P. Devmurari et al. Simultaneous Spectrophotometric determination of lamivudine and Abacavir in the mixture. International Journal of Pharmaceutical Sciences and Research ISSN: 0975-8232IJPSR (2010), Vol. 1, Issue 7.
23. Kokkula Sandhya et al. RP-HPLC Method Development and Validation For Simultaneous Estimation Of LAMIVUDINE, ZIDOVUDINE AND NEVIRAPINE In Tablet Dosage Form. Indo American Journal of Pharmaceutical Research, 2014 ISSN NO: 2231-6876 Vol- 4, Issue 04, 2014.
24. K. Anandakumar et al. RP-HPLC method for simultaneous estimation of lamivudine, tenofovir disoproxil fumarate and efavirenz in tablet formulation. Journal of Analytical Chemistry September 2013, Volume 68, Issue 9, Pg 815-821.
25. Alain Gras et al. Simultaneous quantification of zidovudine, stavudine, lamivudine and nevirapine by Micellar Electrokinetic Capillary Chromatography. Annales De Toxicologie Analytique 2012; 24(4): 177-183.
26. C. Parthiban et al. Simultaneous Estimation and Validation for Determination of Lamivudine and Zidovudine in Human Plasma By LCMS/MS Method. E-Journal of Chemistry Volume 9 (2012), Issue 2, Pages 598-607.
27. M. Ramya Krishna et al. Simultaneous Determination Of Stavudine And Lamivudine In Pharmaceutical Dosage Forms By RP –HPLC. Asian Journal of Pharmaceutical and Clinical Research, Vol 6, Issue 2, 2013, pg.no: 26-29.
28. 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 pg.no: 232-238.
29. Noel A. Gomes et al. LC–MS–MS Method for Simultaneous Analysis of Abacavir and Lamivudine in Human Plasma, and Its Application to a Bioequivalence Study. Chromatographia October 2008, Volume 68, Issue 7-8, Pg 541-550 Date: 29 Aug 2008.
30. G. Sravan Kumar Reddy et al. A New, Simple, Sensitive, Accurate & Rapid Analytical Method Development & Validation For Simultaneous Estimation Of Lamivudine, Abacavir & Zidovudine In Tablet Dosage Form By Using UPLC. International Journal of Pharmaceutical Sciences and Research IJPSR, 2014; Vol. 5(issue-9): 3852-3863.
31. Yadavalli Rekha et al. Method Development and Validation for the Simultaneous Estimation of Efavirenz, Lamivudine and Zidovudine through Stability indicating RPHPLC Method. Research Journal of Pharmaceutical Sciences ISSN 2319 – 555X Vol. 2(issue-4), pg.no: 10-18, May (2013)
32. Palani Venkatesh et al. 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.
33. Sockalingam Anbazhagan et al. Simultaneous quantification of stavudine, lamivudine and nevirapine by UV spectroscopy, reverse phase HPLC and HPTLC in tablets. Journal of Pharmaceutical and Biomedical Analysis Volume 39, Issues 3–4, 15 September 2005, Pages 801–804.
34. Kathryn B Kenney et al. Simultaneous determination of zidovudine and lamivudine in human serum using HPLC with tandem mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis Volume 22, Issue 6, July 2000, Pages 967–983.