The Bioscan AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES 20(4): 788-806,2025 www.thebioscan.com

A new stability indicating UPLC-MS/MS methods for the simultaneous estimation of Abacavir, Dolutegravir and Lamivudine in pharmaceutical dosage forms

***Asia and Revu Baby Nalanda**

Department of Pharmaceutical Analysis

Gandhi Institute of Technology and Management (Deemed to be University),

GITAM School of Pharmacy, Visakhapatnam, India-530045

*Corresponding author: ashuasia26@gmail.com

DOI: 10.63001/tbs.2025.v20.i04.pp788-806

Received on:

10-09-2025

Accepted on:

19-11-2025

Published on:

03-12-2025

ABSTRACT

Abacavir, Dolutegravir and Lamivudine are anti-viral drugs. A new stability indicating LC-ESI-MS method has been proposed and validated for the quantification of Abacavir, Dolutegravir and Lamivudine in pharmaceutical formulations as per ICH guidelines. WATERS, Quattro Premier XE Mass Spectrophotometer (MS/MS) with Software: Mass Lynx, Ver 4.1 and WATERS, Acquity UPLC with PDA detector was employed for the present study. A mixture of 0.2% Formic acid: Acetonitrile was used as mobile phase on gradient mode using Phenomenex Gemini C18, 50*4.6mm*5um with 0.5mL/min and run time of 3.5 min. A mixture of Acetonitrile: Water (80:20) was used as diluent. Linearity was observed over the concentration range 10.0-150ng/ml for Abacavir, Dolutegravir and Lamivudine by the proposed method and the methods are found to be simple, precise and accurate. Forced degradation studies were performed and the method is found to be selective and specific.

INTRODUCTION

Abacavir1 (CAS no. 136470-78-5) is chemically ((1S,4R)-4-(2-amino-6-(cyclopropyl amino)-9H-purin-9-yl) cyclopent-2-en-1-yl) methanol, which is an FDA approved antiretroviral drug with molecular formula, C14H18N6O and molecular weight 286.332 grams/mole (Figure 1A). Dolutegravir sodium2 is chemically, sodium (4R, 12aS)-9-[(2,4-difluorobenzyl) carbamoyl]-4methyl6, 8- dioxo-3, 4, 6, 8, 12, 12a- hexahydro- 2H pyrido [1', 2':4, 5] pyrazino[2,1-b] [1,3] oxazin 7-olate (Figure 1B). Lamivudine3 is chemically 4-amino-1- [(2R, 5S)-2- (hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one (Figure 1C). The combination of Abacavir, Dolutegravir and Lamivudine is used for the treatment of HIV viral infection4.

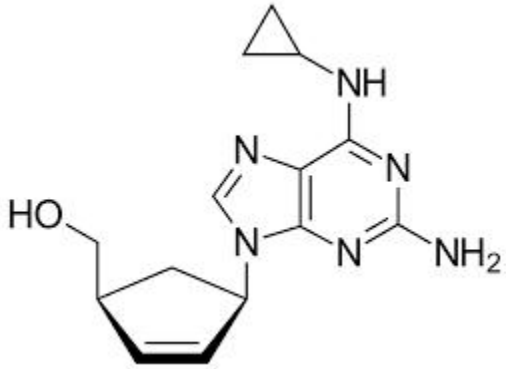


Figure 1A: Chemical structure of Abacavir (AB)

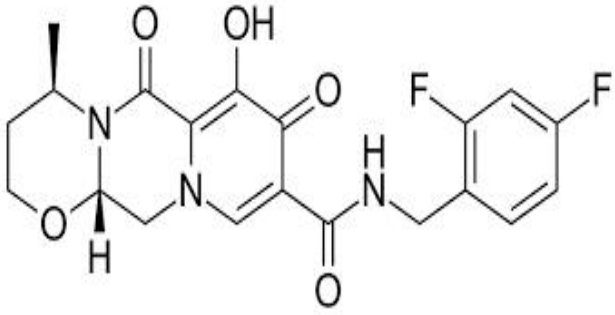


Figure 1B: Chemical structure of Dolutegravir (DL)

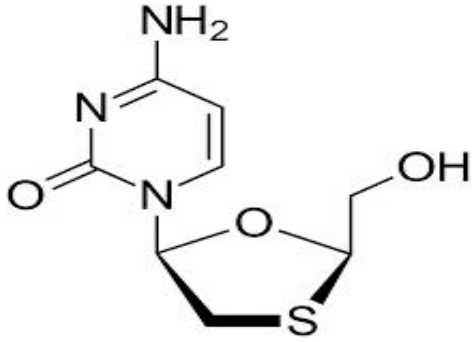


Figure 1C: Chemical structure Lamivudine (LM)

The combination of Abacavir, Dolutegravir and Lamivudine was studied by different authors using different columns and different mobile phase compositions and in the present study a new stability indicating UPLC-MS/MS method has been developed and validated for the estimation of Abacavir, Dolutegravir and Lamivudine and the method was validated as per ICH guidelines.

Narottam Pal *et al.,* have developed a HPLC

method for the simultaneous estimation of Lamivudine, Abacavir and Dolutegravir in combined dosage form with their stability studies

using polar column, Kromasil and a mobile phase mixture consisting of buffer and acetonitrile (65:35) with a flow rate of 1 mL/min (Isocratic mode) and UV detection at 257 nm and Lamivudine, Abacavir and Dolutegravir were eluted at 2.250 min, 2.734 min and 9.633 min respectively with linearity 15-90 µg/mL, 30-180 µg/mL and 2.5-15 µg/mL.

China Babu *et al.,* have developed a stability indicating RP-HPLC method for the simultaneous estimation of Abacavir, Dolutegride and Lamivudine in bulk and pharmaceutical formulation using Agilent TC-C18 column and a mobile phase mixture consisting of Methanol: Water (70:30) with a flow rate of 1 mL/min (Isocratic mode) and UV detection at 257 nm where Abacavir, Dolutegravir and Lamivudine were eluted at be 2.6, 2.9 and 6.6 min respectively with linearity 150-450 µg/mL, 12.5-37.5 µg/mL and 75-225 µg/mL respectively.

Nagaraju *et al.,* have developed a RP-HPLC method for the simultaneous estimation of Abacavir, Dolutegravir and Lamivudine in active pharmaceutical ingredients and marketed tablet formulation using Lichrosphere RP C8 column and a mobile phase mixture consisting of Ethanol: Ethyl acetate (80:20) with a flow rate of 1 mL/min and UV detection at 260 nm where Abacavir, Dolutegravir and Lamivudine were eluted at be 2.31, 3.120 and 4.59 min respectively with linearity 40-130 µg/mL.

MATERIALS AND METHODS

Instrumentation

WATERS, Quattro Premier XE Mass Spectrophotometer (MS/MS) with Software: Mass Lynx, Ver 4.1 and WATERS, Acquity UPLC with PDA detector was employed for the present study. A mixture of 0.2% Formic acid: Acetonitrile was used as mobile phase on gradient mode with 0.5 mL/min with run time 3.5 min and a mixture of Acetonitrile: Water (80:20) was used as diluent. The column temp was maintained at 40°C and that of the sample temp was maintained at 10°C.

Preparation of stock solution

2.0 mg of each of Abacavir, Dolutegravir and Lamivudine were weighed individually and dissolved in 2mL of DMSO to get 1mg/mL of stock solutions. A mixture of 80 mL of Acetonitrile and 20 mL water was used as

diluent throughout the work. 20 uL of each of Abacavir, Dolutegravir and Lamivudine stock solutions were taken and 1980 uL of diluent was added to get 10 ug/mL (Intermediate stock).

A mixed stock solution was prepared by mixing 200 ul of Abacavir (10 ug/mL), 200 ul of Dolutegravir (10 ug/mL) and 200 ul of Lamivudine (10 ug/mL) with 1400 uL of diluent to get 1.0 ug/mL of mixed stock and from this 100 ul was taken and 900 uL of diluent was added to get 100 ng/mL and the resulting solution was sonicated for 30 mins.

Method validation

Linearity, Precision, Accuracy and Robustness

10.0-150 ng/ml Abacavir, Dolutegravir and Lamivudine solutions were prepared from the stock solution and diluted with the mobile phase and each solution was injected (n=3) into the UPLC/MS/MS system and the area response was noted. A calibration graph was drawn by plotting the concentration of the each of Abacavir, Dolutegravir and Lamivudine drug solutions on the x-axis and the corresponding peak response of the chromatograms on the y-axis. The precision and accuracy studies were conducted by spiking and the percentage recovery as well as % RSD were calculated from the regression equation.

Forced degradation studies[9]

A standard solution was prepared by mixing 200 uL (10 ug/mL (Intermediate stock)) of each drug and 1800 uL diluent was added to get 1 ug/mL conc.

For acidic degradation study, a solution was prepared by mixing 10% (200uL (10 ug/mL (Intermediate stock)), 10% (i. e 200 ul 1N HCl) and 1600 diluent to get 1 ug/mL conc. For basic degradation study, a solution was prepared by mixing 10% (200 uL (10 ug/mL (Intermediate stock)), 10% (i. e 200 ul 10% NaOH) and 1600 diluent to get 1 ug/mL conc. For water degradation study, a solution was prepared by mixing 10% (200 uL (10 ug/mL (Intermediate stock)), 10% (i. e 200 ul water) and 1600 diluent to get 1 ug/mL conc. For peroxide degradation study, a solution was prepared by mixing 10% (200 uL (10 ug/mL (Intermediate stock)), 10% (i. e 200 ul 3% H_2O_2) and 1600 diluent to get 1 ug/mL conc. For thermal degradation study, a solution was prepared by mixing 200 uL (10 ug/mL (Intermediate stock)) respectively for each drug and 1800 diluent to get 1 ug/mL conc. For photolytic degradation study, a solution was prepared by mixing 200 uL (10 ug/mL (Intermediate stock)) respectively for each drug

and 1800 diluent to get 1 ug/mL conc. The same procedure was continued for all the three compounds: Abacavir, Dolutegravir and Lamivudine and the gradient program with (Column Temp 40ºC; Sample temp 10ºC) run time 3.50 mins was given below.

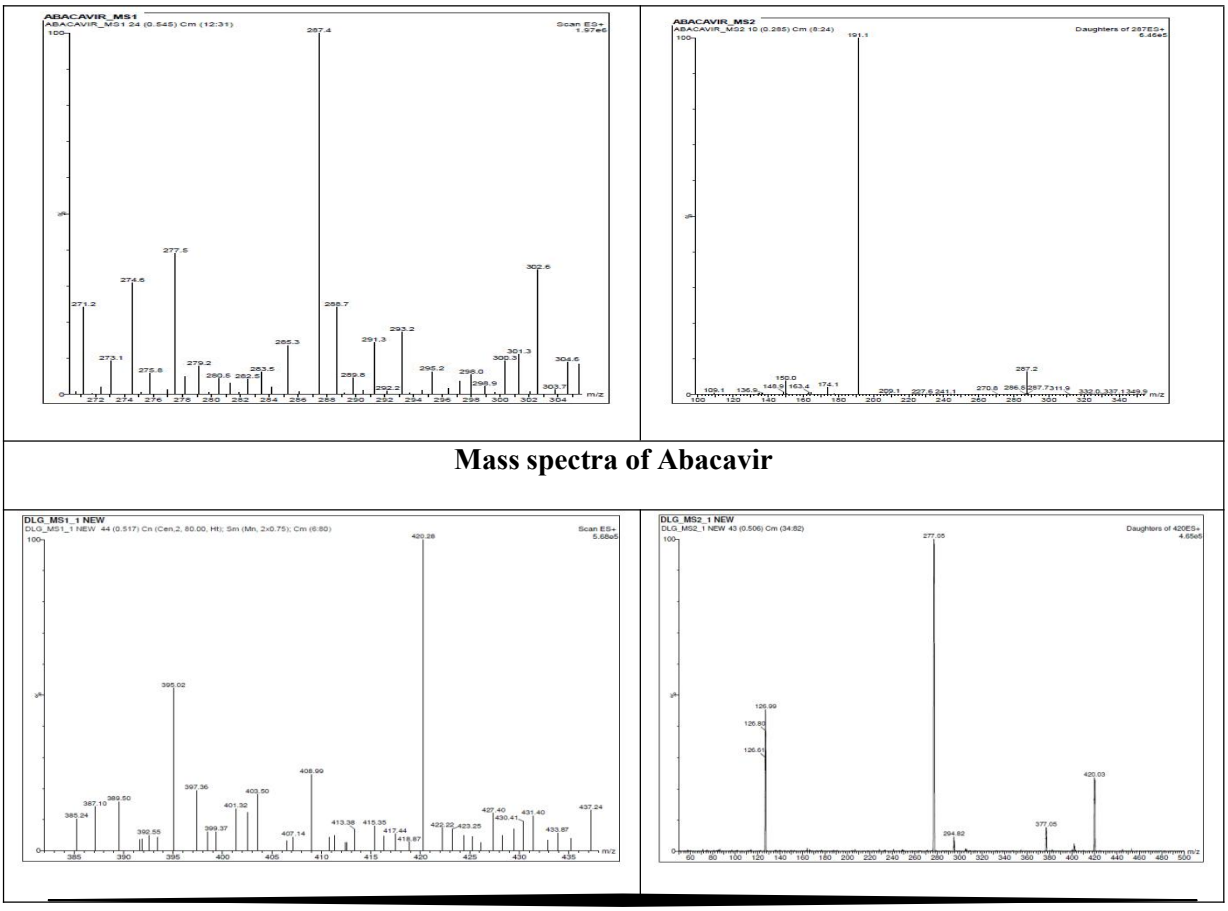
RESULTS AND DISCUSSION

A new stability-indicating UPLC/MS/MS method has been proposed for the quantification of the combined formulation of Abacavir, Dolutegravir and Lamivudine. The previously reported methods were reviewed with the present proposed method in Table 1. WATERS, Quattro Premier XE Mass Spectrophotometer (MS/MS) with Software: Mass Lynx, Ver 4.1 and WATERS, Acquity UPLC with PDA detector was used for the present study on gradient mode (Table 2)

Table 1: Literature survey

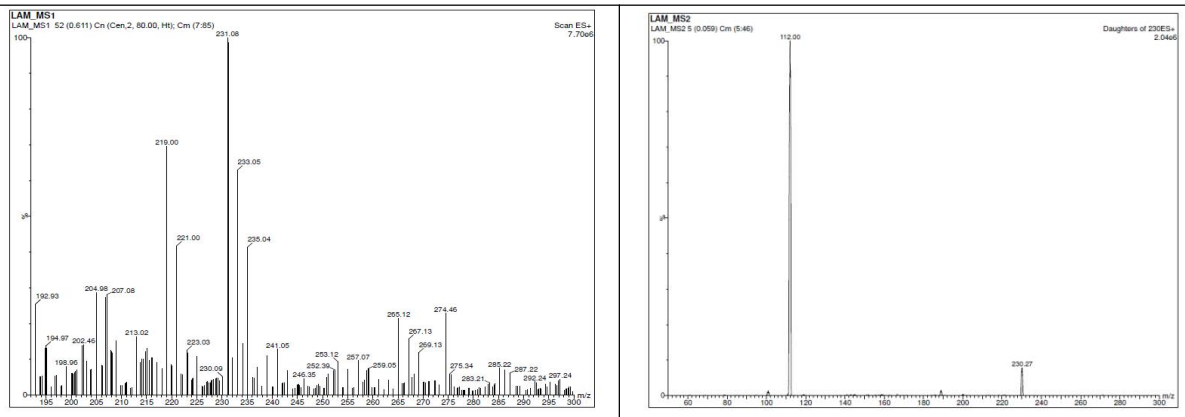
Method	Column	Mobile phase (v/v)	λ (nm)	Rt (min)	Linearity (µg/mL)	Ref
RP-HPLC (Isocratic mode)	Kromasil	Buffer: Acetonitrile (65:35)	257	2.734 (AB) 9.633 (DL) 2.250 (LM)	30-180 (AB) 2.5-15 (DL) 15-90 (LM)	5
RP-HPLC (Isocratic mode)	Agilent TC-C18	Methanol: Water (70:30)	257	2.6 (AB) 2.9 (DL) 6.6 (LM)	150-450 (AB) 12.5-37.5 (DL) 75-225 (LM)	6
	Lichrosphere RP C8	Ethanol: Ethyl acetate (80:20)	260	2.31 (AB) 3.120 (DL) 4.59 (LM)	40-130	7
UPLC-MS/MS	Phenomenex Gemini 5um C18 50*4.6mm	0.1% Formic acid: Acetonitrile (Gradient mode)		2.31 (AB) 3.120 (DL) 4.59 (LM)	10.0-150 ng/mL	Present method

The mass spectra of Abacavir, Dolutegravir and Lamivudine obtained in the optimized chromatographic conditions were shown in Figure 2.



Mass spectra of Abacavir

Mass spectra of Dolutegravir



Mass spectra of Lamivudine

Figure 2: Mass spectra of Abacavir, Dolutegravir and Lamivudine

Linearity, Precision and accuracy

Abacavir, Dolutegravir and Lamivudine solutions obeys Beer-Lambert's law over the concentration range 10.0-150 ng/ml (Table 3) and the linear regression equation was found to be y = 0.9053x + 5.3384 (R^2 = 0.993), y = 0.9145x + 4.5544 (R^2 = 0.9923) and y = 0.9434x + 3.5387 (R^2 = 0.9923) (Figure 3).

Table 3: Linearity

Conc. (ng/mL) (ppb)	*Mean peak area		
	AB	DL	LM
10	9.578	9.47	10.097
25	26.844	27.608	22.9
50	55.988	56.648	56.367
75	75.164	74.991	77.954
100	97.268	94.719	98.204
125	120.985	113.101	124.394
150	135.887	144.576	139.558

*Mean of three replicates

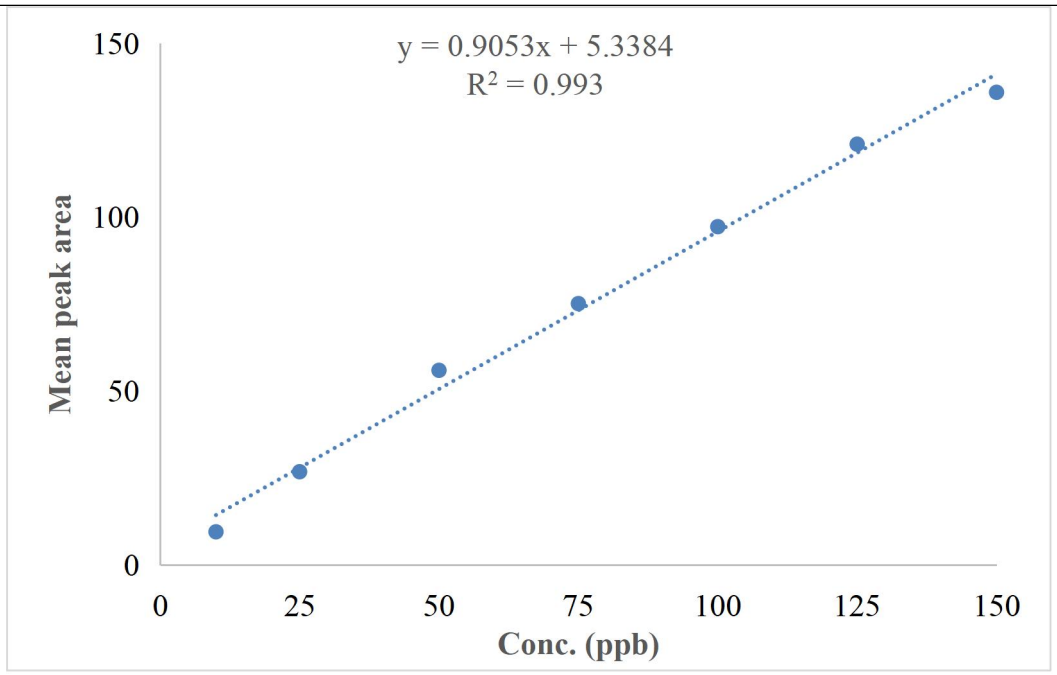


Figure 3A: Calibration curve of Abacavir

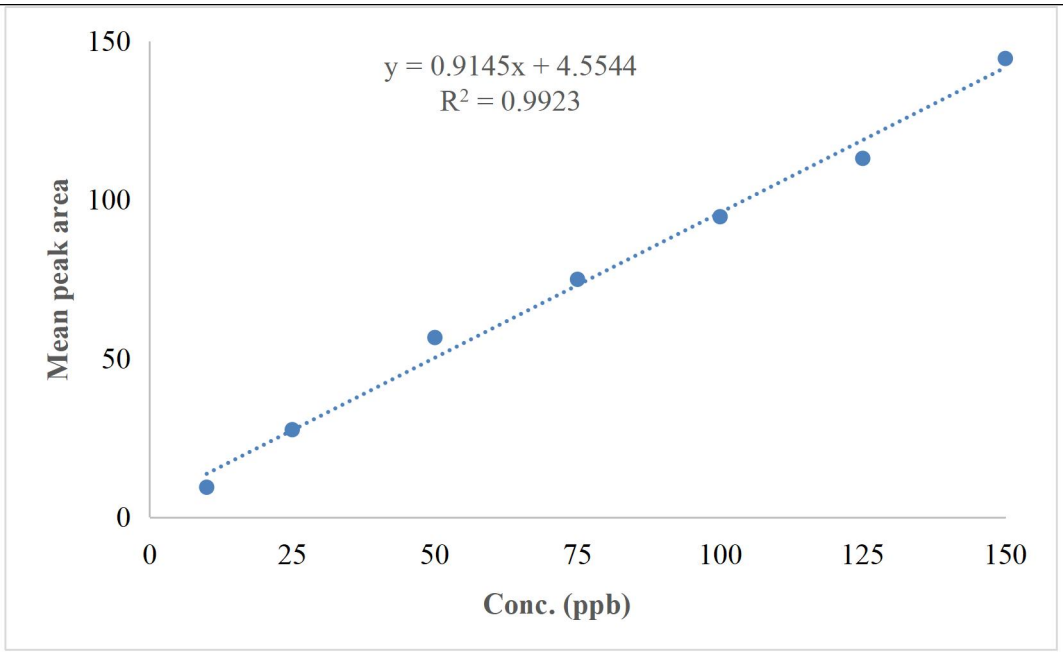


Figure 3B: Calibration curve of Dolutegravir

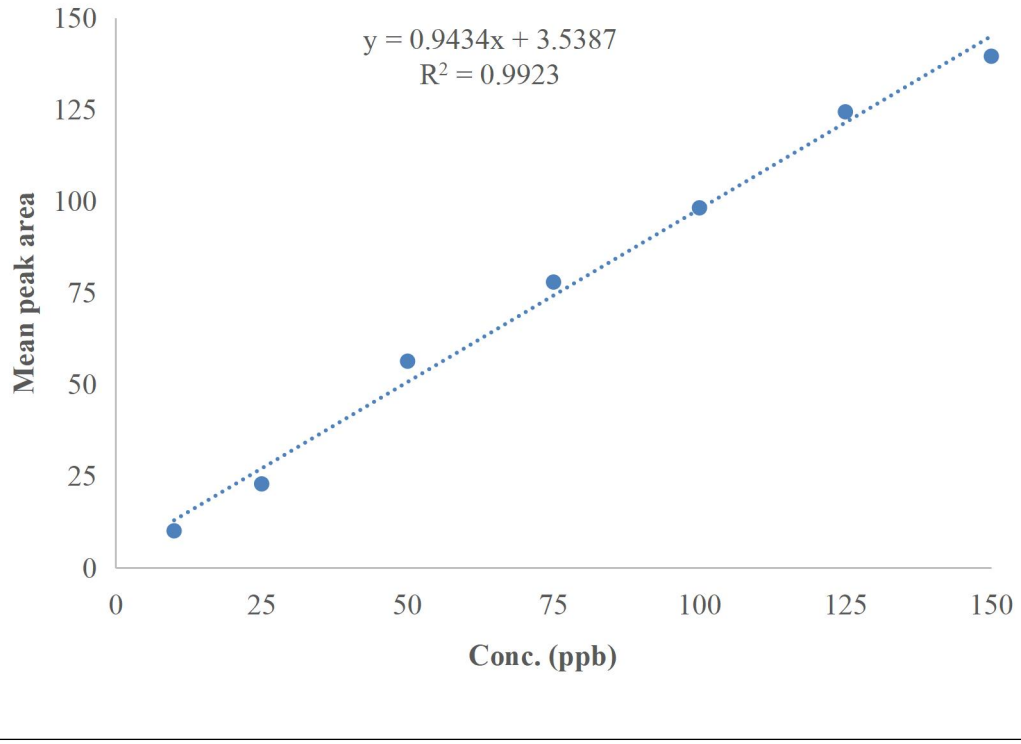


Figure 3C: Calibration curve of Lamivudine

Table 4: Precision study

Conc. (ng/ml)	*Area response		
	Abacavir	Dolutegravir	Lamivudine
10	11673	21566	4800
10	10880	20967	4340
10	10321	19599	4608
10	11131	19648	4569
10	10776	19067	4490
10	11380	19610	4482
***Mean peak area ± SD (% RSD)**	11026.8 ± 476.61 (4.32)	20076.167 ± 965.39 (4.81)4548.1667 ± 153.97 (3.39)	4548.1667 ± 153.97 (3.39)

*Mean of three replicates

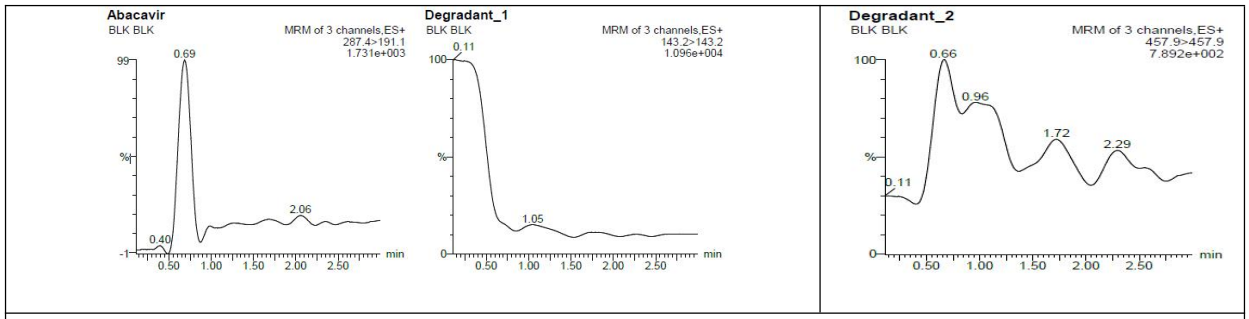
Table 5: Accuracy study

Level	*Area response			% Accuracy		
	Abacavir	Dolutegravir	Lamivudine	Abacavir	Dolutegravir	Lamivudine
50 %	5582.667	9933.66667	2240.67	50.62801	49.4798	49.4798
100 %	11016.67	19799.6667	4491	99.9078	98.6228	98.6227
150 %	16347.33	29806.6667	6775.33	148.2505	148.4679	148.4679

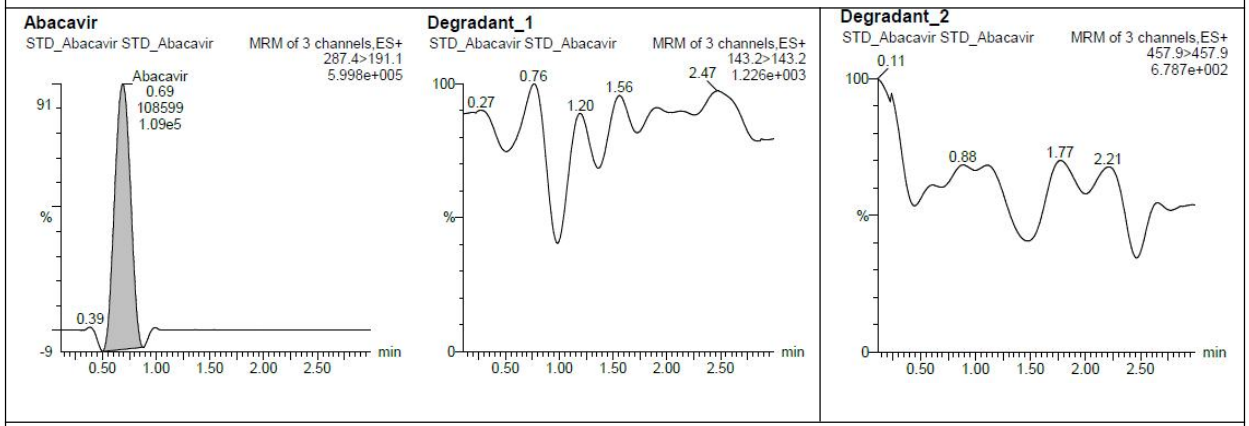
*Mean of three replicates

Forced degradation studies

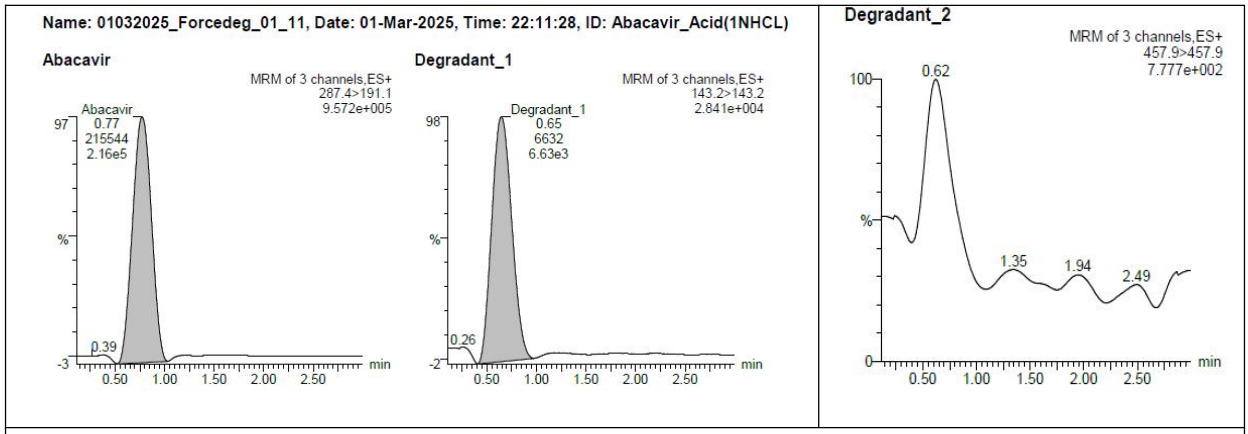
Abacavir, Dolutegravir and Lamivudine were exposed to different stress conditions under the optimized chromatographic conditions and then injected in to the system. The corresponding chromatograms (MRM) were shown in Figure 4, Figure 5 and Figure 6.



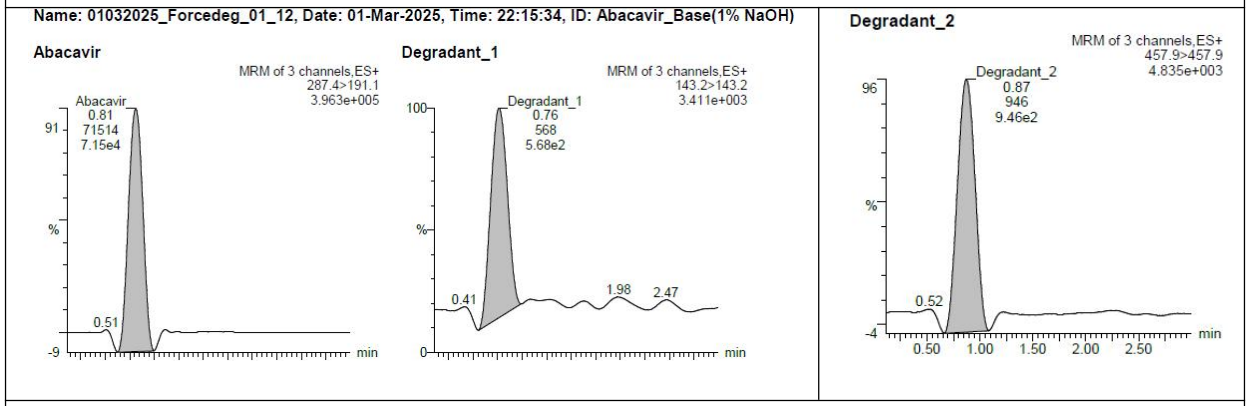
Blank



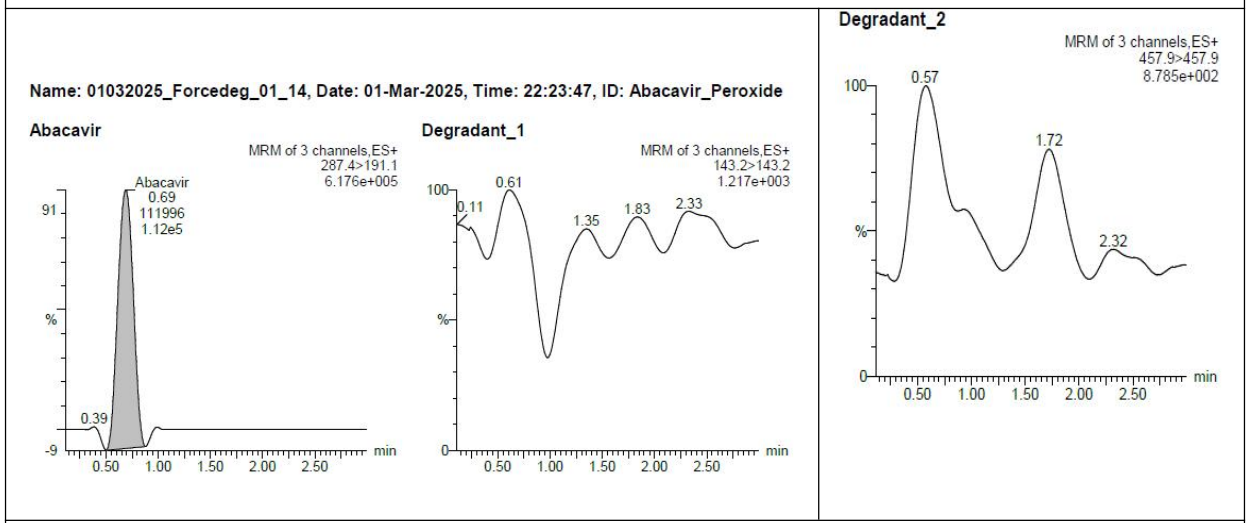
Abacavir standard



Abacavir (Acidic degradation)



Abacavir (Basic degradation)



Abacavir (Oxidative degradation)

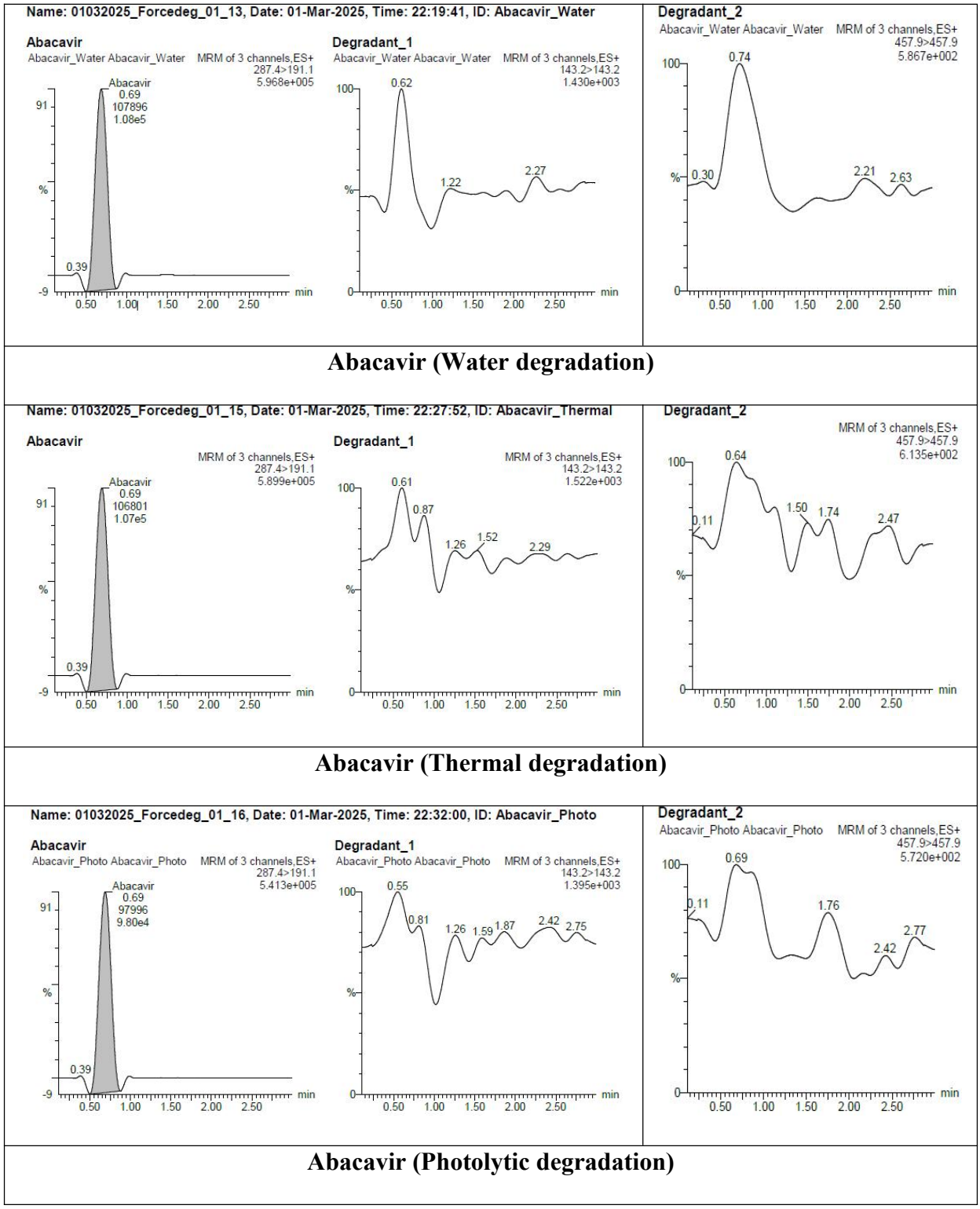
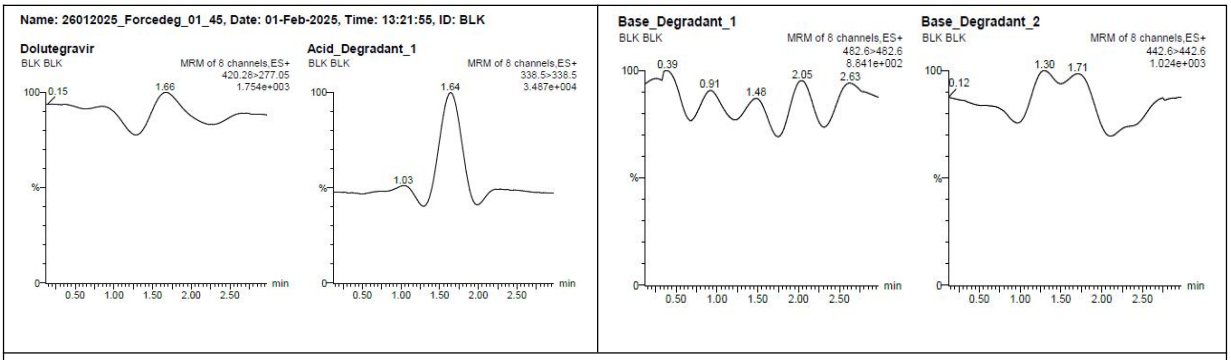
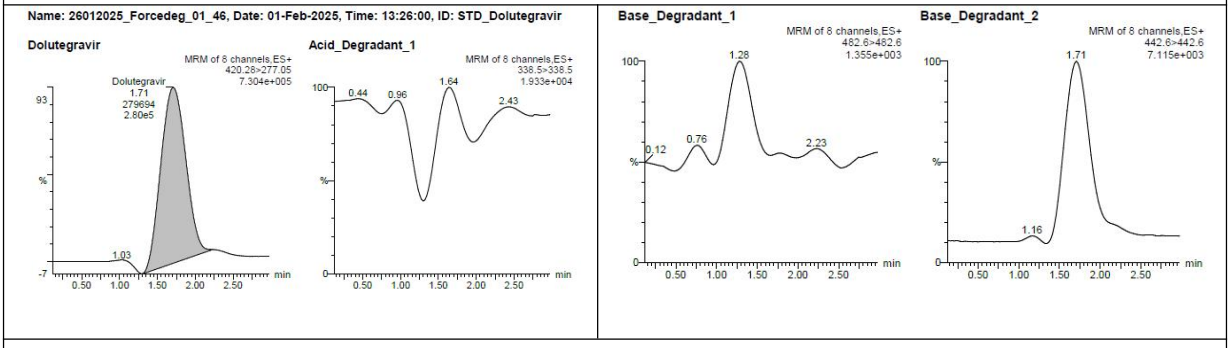


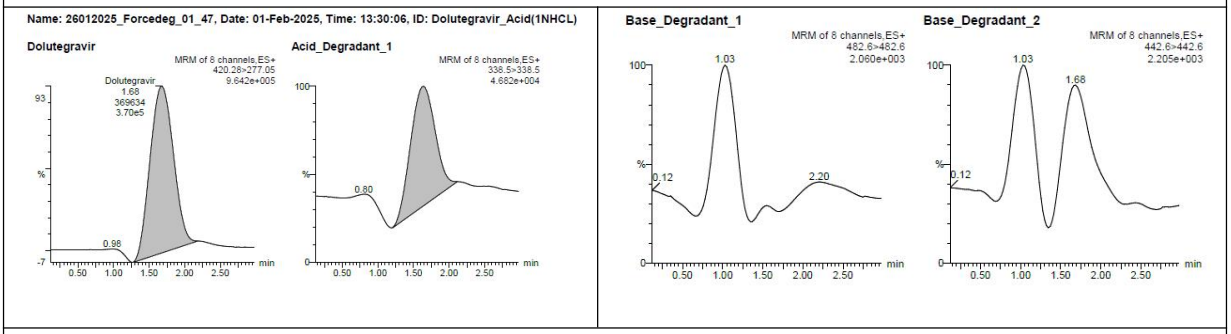
Figure 4: UPLC/MS/MS chromatograms of Abacavir (Forced degradation studies)



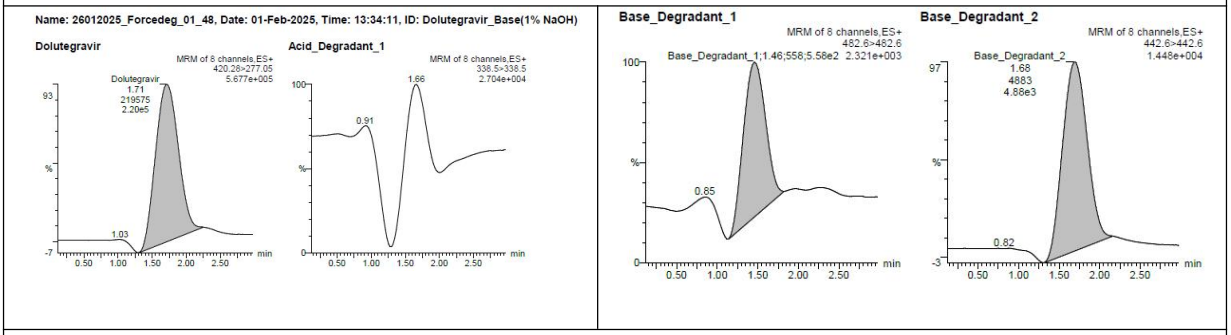
Blank



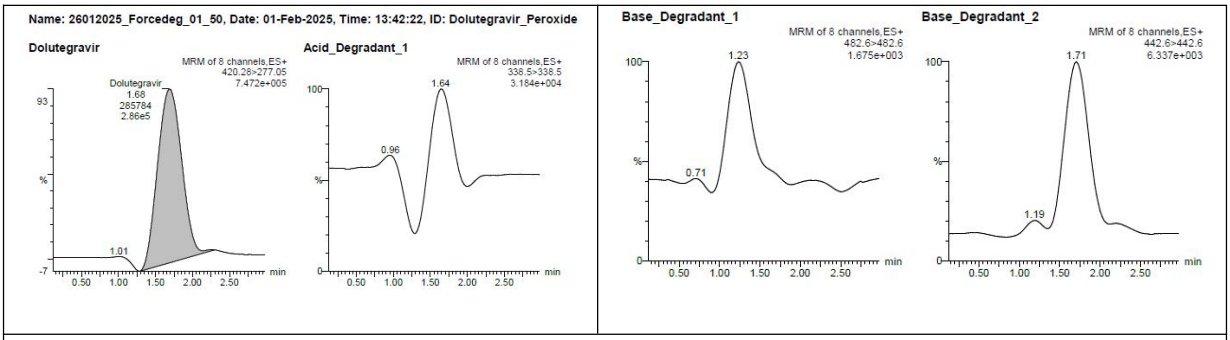
Dolutegravir standard



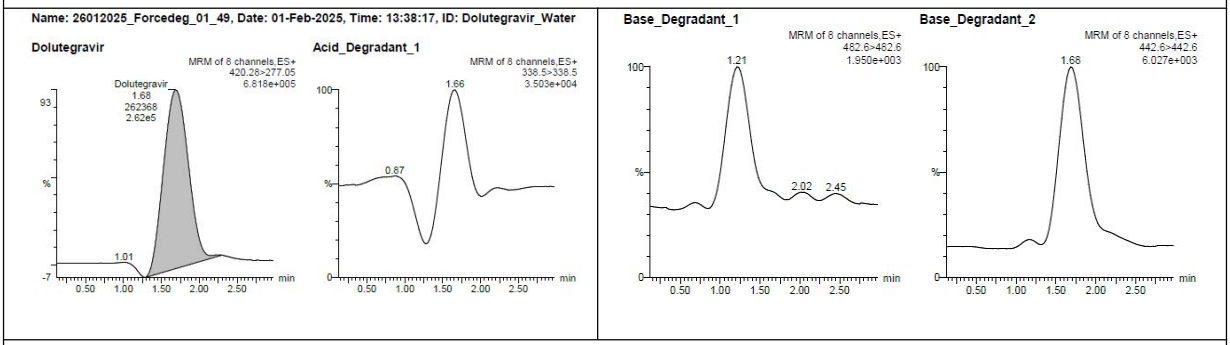
Dolutegravir (Acidic degradation)



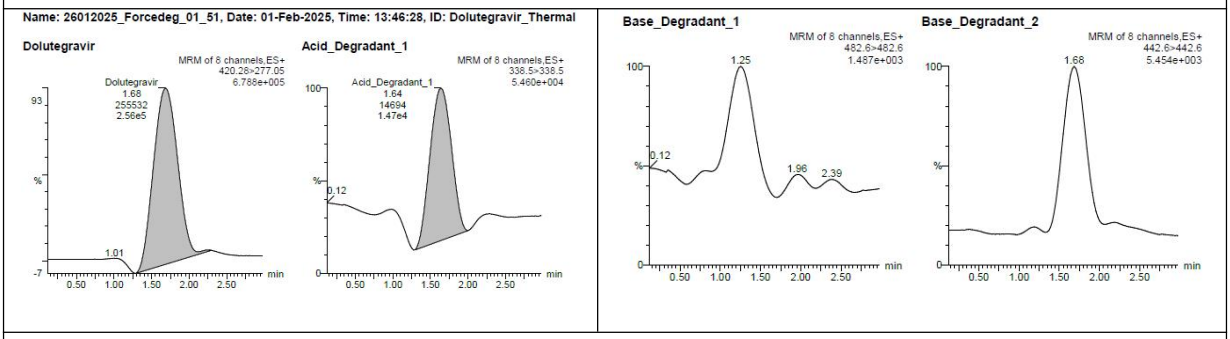
Dolutegravir (Basic degradation)



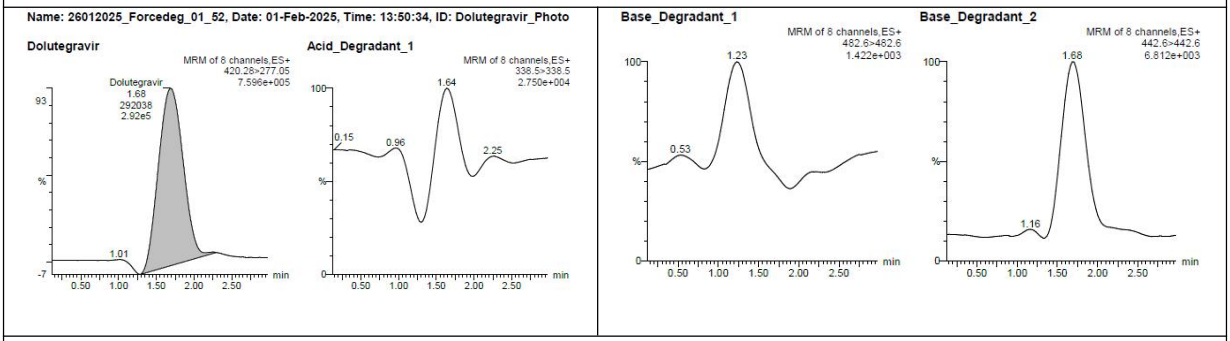
Dolutegravir (Oxidative degradation)



Dolutegravir (Water degradation)

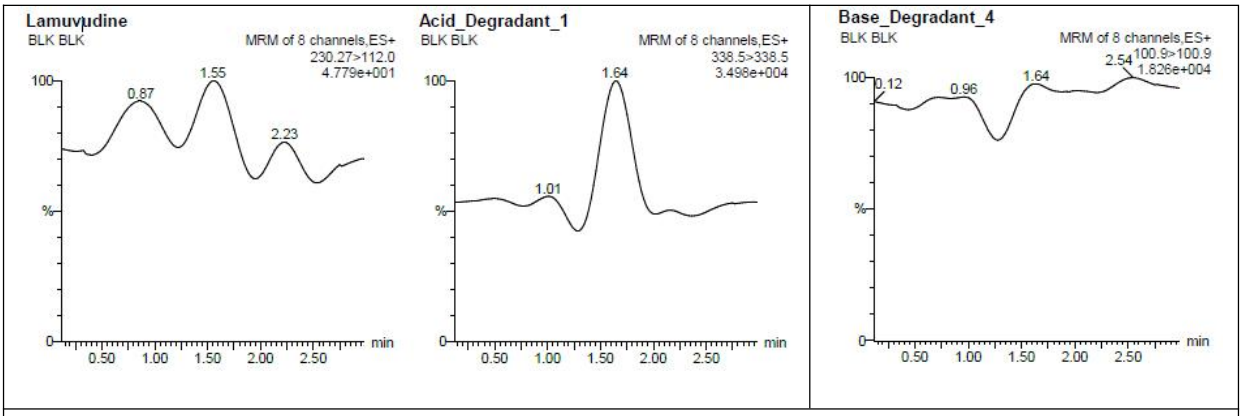


Dolutegravir (Thermal degradation)

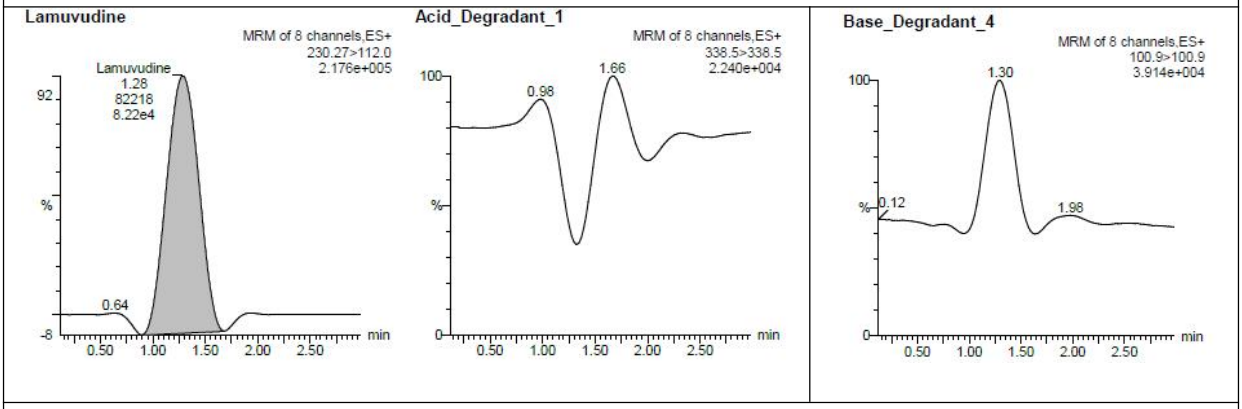


Dolutegravir (Photolytic degradation)

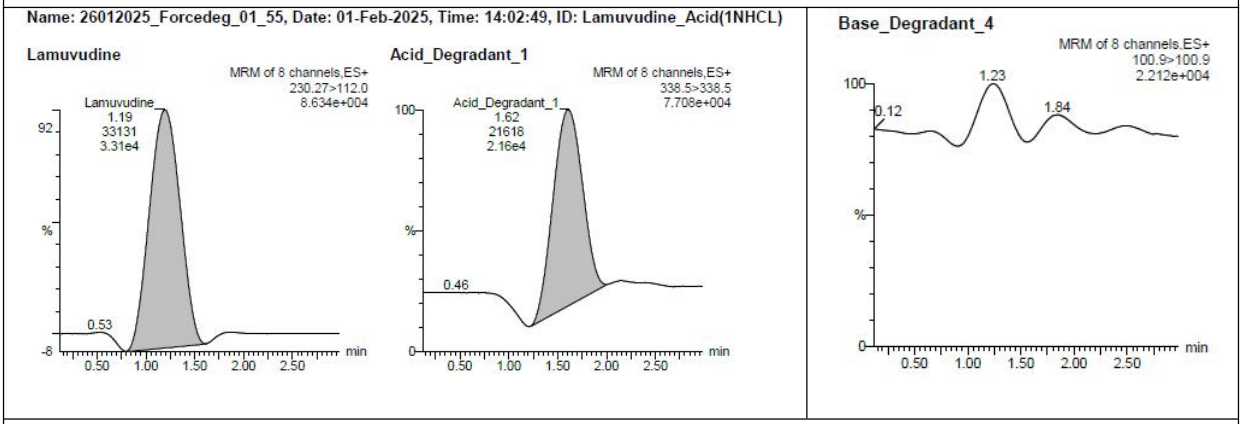
Figure 5: UPLC/MS/MS chromatograms of Dolutegravir (Forced degradation studies)



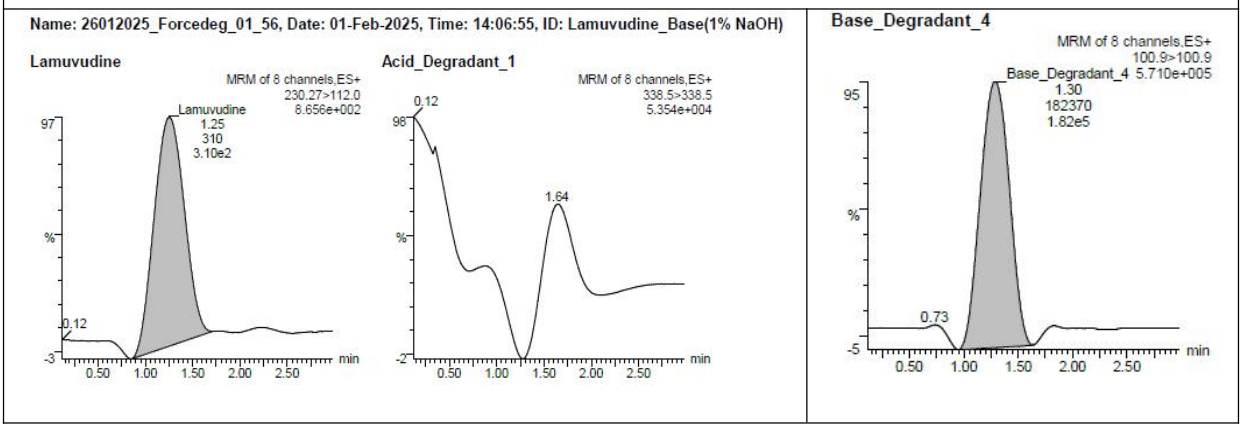
Blank



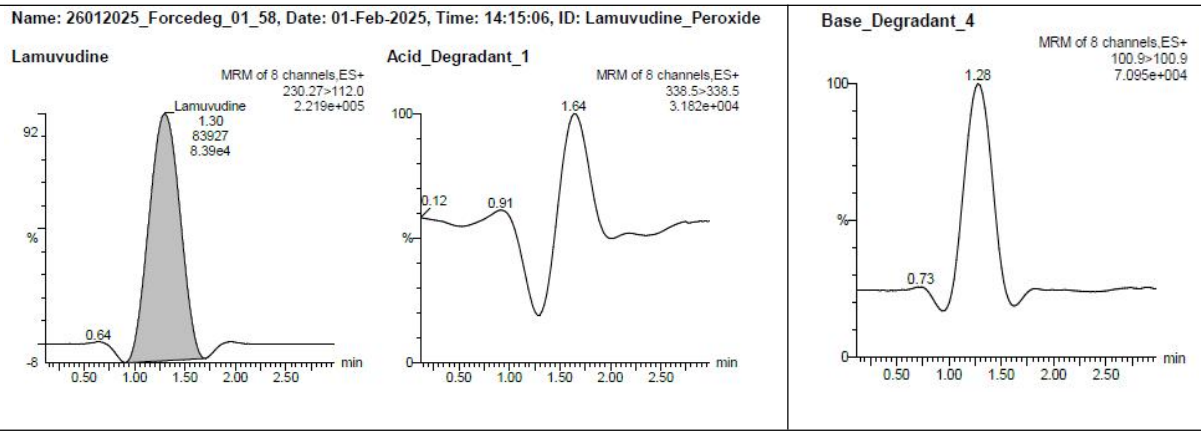
Lamivudine standard



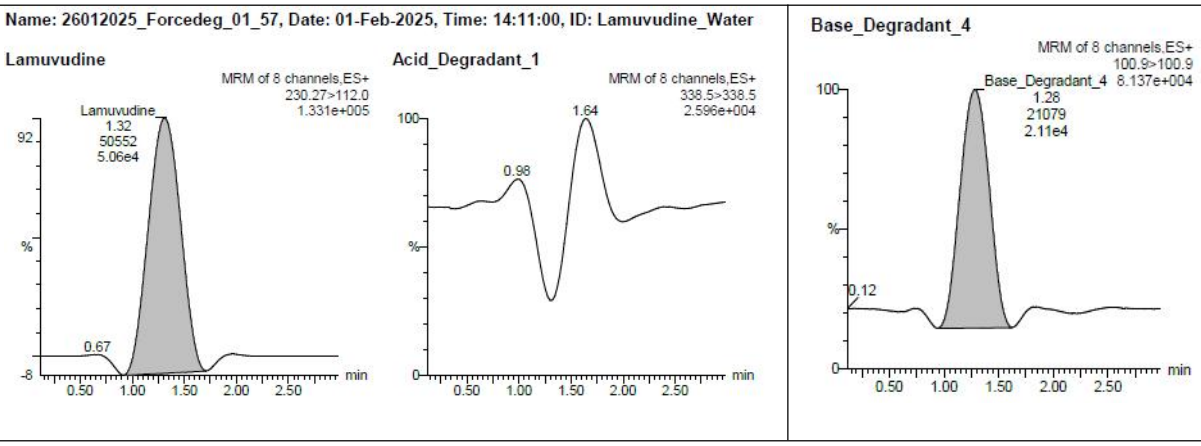
Lamivudine (Acidic degradation)



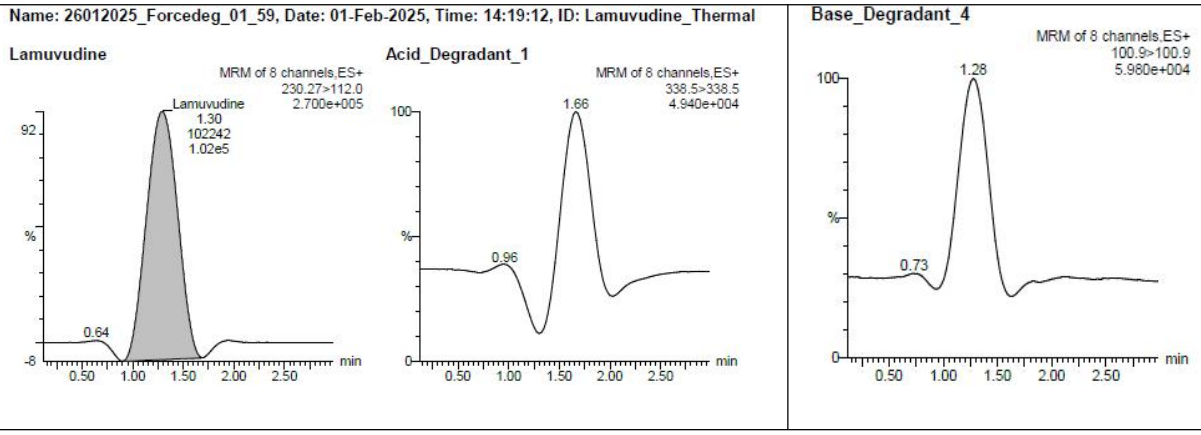
Lamivudine (Basic degradation)



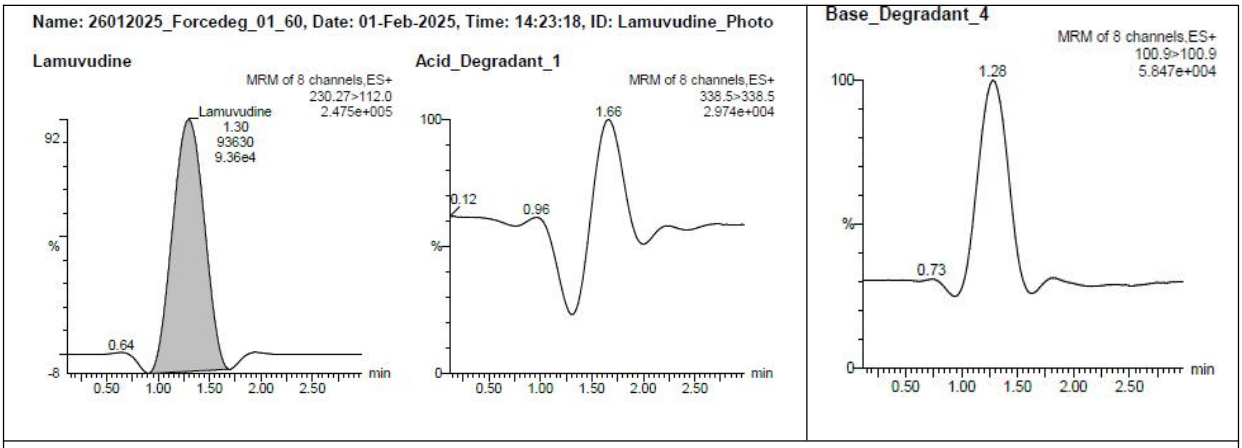
Lamivudine (Oxidative degradation)



Lamivudine (Water degradation)



Lamivudine (Thermal degradation)



Lamivudine (Photolytic degradation)

Figure 6: UPLC/MS/MS chromatograms of Lamivudine (Forced degradation studies)

The details of the forced degradation studies of Abacavir, Dolutegravir and Lamivudine were shown in Table 6.

Table 6: Forced degradation studies

Sample	Stress condition	Area of standard			Total degraded		
		Abacavir	Dolutegravir	Lamivudine	Abacavir	Dolutegravir	Lamivudine
Standard	Control	108599	279694	82218	-	-	-
Acid	1N HCl / 7 days	101967	267060	60600	6.11	4.52	26.29
Base	1% NaOH / 7 days	107085	274253	320	1.39	1.75	99.61
Water	Water / 7 days	108599	279694	61139	-	-	25.64

Peroxide	3% H_2O_2 / 7 days	108599	279694	82218	-	-	-
Thermal	70ºC / 3 Hrs	108599	265000	82218	-	5.25	-
Photo	12000 / 7 days	108599	279694	82218	-	-	-

*Mean of three replicates

CONCLUSION

The authors have established a new stability indicating UPLC-MS/MS method for the estimation of Abacavir, Dolutegravir and Lamivudine. The method is simple, precise and accurate and used for the routine analysis of Abacavir, Dolutegravir and Lamivudine pharmaceutical formulations and no interference of excipients was observed during the assay.

ACKNOWLEDGEMENT

The authors declare no conflict of interest.

REFERENCES

1. Yuen GJ, Weller S and Pakes GE. A review of the pharmacokinetics of abacavir". Clinical Pharmacokinetics. 2008; 47 (6): 351–371.

2. Katlama C and Murphy R. Dolutegravir for the treatment of HIV. Expert Opin Investig Drugs. 2012; 21(4): 523-530.

3. Roussos A, Koilakou S, Kalafatas I, Kalantzis C, Apostolou N, Grivas E, Raptis N and Mantzaris G. Lamivudine treatment for acute severe hepatitis B: Report of a case and review of the literature. Acta Gastroenterol Belg. 2008; 71(1): 30-32.

4. Walmsley S, Baumgarten A, Berenguer J, Felizarta F, Florence E, Khuong-Josses MA, Kilby JM, Lutz T, Podzamczer D, Portilla J, Roth N, Wong D, Granier C, Wynne B and Pappa K. Brief Report: Dolutegravir Plus Abacavir/Lamivudine for the treatment of HIV-1 infection in antiretroviral therapy-

naive patients: Week 96 and week 144 results from the single randomized clinical trial. J Acquir Immune Defic Syndr. 2015; 70(5): 515-519.

5. Narottam Pal, Srinivasa Rao A and Ravikumar P. Simultaneous HPLC method development and validation for estimation of Lamivudine, Abacavir and Dolutegravir in combined dosage form with their stability studies. Asian Journal of Chemistry. 2016; 28(2): 273-276.

6. China Babu D, Hanumantha Rao K, Sai Sri Harsha G, Alekhya D, Sirisha V, Shailendra Singh Narwariya and Aleesha SK. Simultaneous stability indicating RP-HPLC method development and validation of Abacavir, Dolutegride and Lamivudine in bulk and pharmaceutical formulation. Journal of Chemical Health Risks. 2024; 14(2): 3878-3884.

7. Gollapalli Nagaraju, Ritesh Agarwal and Rama Rao Nadendla. An eco-friendly HPLC method for the simultaneous estimation of Abacavir, Dolutegravir and Lamivudine in active pharmaceutical ingredients and marketed tablet formulation. International Journal of Pharmaceutical Sciences and Research. 2022; 13(9): 3683-3689.

8. ICH Validation of analytical procedures: Text and methodology Q2 (R1), International Conference on Harmonization (2005).

9. ICH Stability testing of new drug substances and products Q1A (R2), International Conference on Harmonization (2003).

1. . Sci Rep. 2021, 11:15853.
2. Rasteau S., Ernenwein D., Savoldelli C., Bouletreau P. Artificial intelligence for oral and maxillo-facial surgery: A narrative review. J. Stomatol. Oral. Maxillofac. Surg. 2022;123:276–282
3. Sillmann YM, Monteiro JLGC, Eber P, Baggio AMP, Peacock ZS, Guastaldi FPS. Empowering surgeons: will artificial intelligence change oral and maxillofacial surgery? Int J Oral Maxillofac Surg. 2025 Feb;54(2):179-190.