SIMPLE RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ABACAVIR AND LAMIVUDINE IN TABLET FORMULATION

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ABSTRACT : The objective of the present research work was to develop an innovative, simple, and economic method for estimation of Abacavir and Lamivudine in bulk and dosage form by RP-HPLC.The chromatographic conditions were performed on Waters ODS (C18) RP Column, 250 mm x 4.6 mm. 5µm as stationary phase and mobile phase was prepared ACN & Methanol & Phosphate buffer (0.02M) = 30:35:35 (pH-2.6)and other conditions optimized were: flow rate (1.0 ml/minute), wavelength (284 nm), Run time was maintained at 14 minutes.The analytical method is valid for estimation of Abacavir and Lamivudine over a range of 10 µg/ml–50 µg/ml. The results of system suitability test, linearity, precision and accuracy, robustness, specificity, LOD and LOQ and stabilities presented in this report are within the acceptance range.A specific, sensitive, economic method estimation of Abacavir and Lamivudine has been developed based on ICH Guidelines with bulk and dosage forms.

Key Words: Abacavir and Lamivudine , HPLC, Method Development , ICH, Validation , Accuracy, Precision.

I. INTRODUCTION

Abacavir (ABC) is a powerful nucleoside analogue reverse transcriptase inhibitor (NRTI) used to treat HIV and AIDS. Chemically, it is a synthetic carbocyclic nucleoside and is the enantiomer with 1S, 4R absolute configuration on the cyclopentene ring.

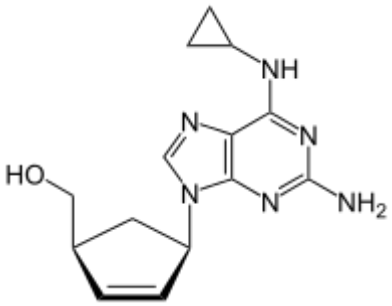

Fig.1. Structure of Abacavir

Lamivudine (2′,3′-dideoxy-3′-thiacytidine, commonly called 3TC) is a potent nucleoside analog reverse transcriptase inhibitor (nRTI). It is marketed by GlaxoSmithKline with the brand names Zeffix, Heptovir, Epivir, and Epivir-HBV.

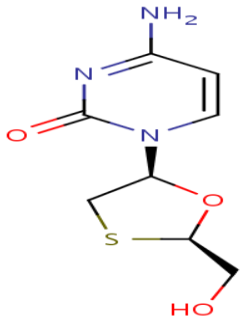

Fig.2. Structure of Lamivudine

II. EXPERIMENTAL

2.1 Materials and Methods:

Pharmaceutical grade working standard Abacavir and Lamivudine were obtained from Syncorp Pvt. Laboratories, Hyderabad, India. All chemicals and reagents were HPLC grade and were purchased from S D Fine-Chem Limited & Loba Chemie Pvt Ltd, Mumbai, India.

2.2 Instrumentation:

The analysis was performed using HPLC (Waters-717 series) with PDA detector and data handling system EMPOWER2 software, UV-Visible double beam spectrophotometer (ELICO SL-159), analytical balance 0.1mg Sensitivity (SHIMADZU), pH meter (Labindia), ultra sonicator. The column used is Symmetry ODS RP C_{18},5µm, 15mm x 4.6mm i.d. (as Stationary phase) with the flow rate 1.0ml/min (isocratic).

2.3 Sample & Standard Preparation for the Analysis

25 mg of Abacavir and Lamivudine standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.1ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

2.4 Selection of wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent. (After optimization of all conditions) for UV analysis. It is scanned in the UV spectrum in the range of 200 to 400nm. While scanning the Abacavir and Lamivudine solution we observed the maxima at 284nm.

2.5 Method Development

2.5.3 Summary of Optimized Chromatographic Conditions:

The Optimum Chromatographic conditions obtained from experiments can be summarized as below:

Table-1: Summary of Optimised Chromatographic Conditions

Mobile phase	ACN:Methanol : Phosphate buffer (0.02M) = 30:35:35 (pH-2.6)
Column	Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.
Column Temperature	Ambient
Detection Wavelength	284 nm
Flow rate	1.0 ml/ min.
Run time	14 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase

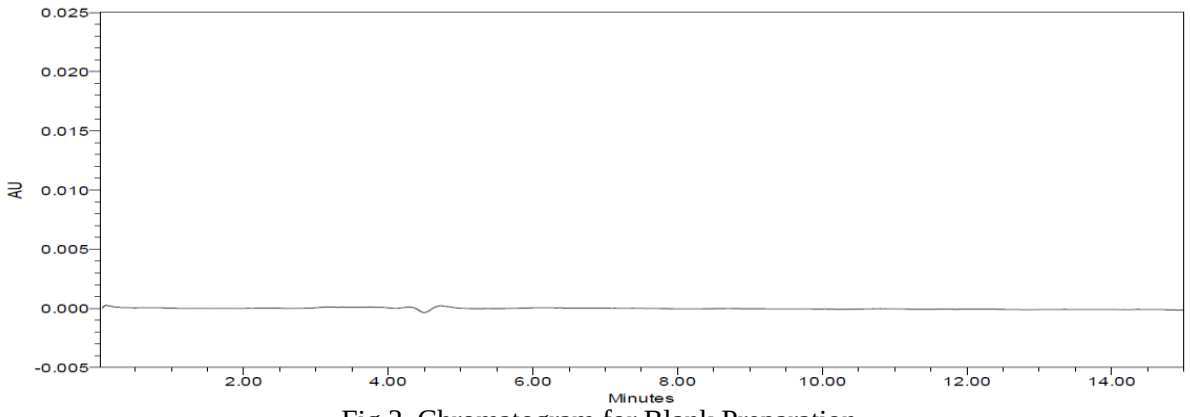

Fig.3. Chromatogram for Blank Preparation

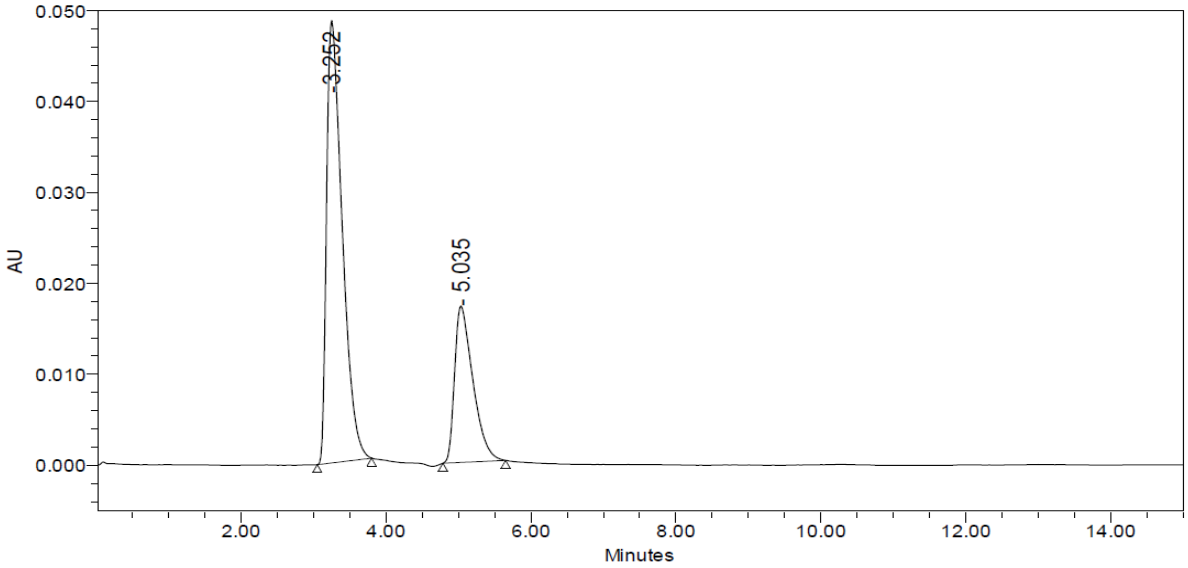

Fig.4. Chromatogram of Abacavir and Lamivudine in Optimized Condition

2.6 Method validation:

2.6.1 Linearity & Range: Abacavir

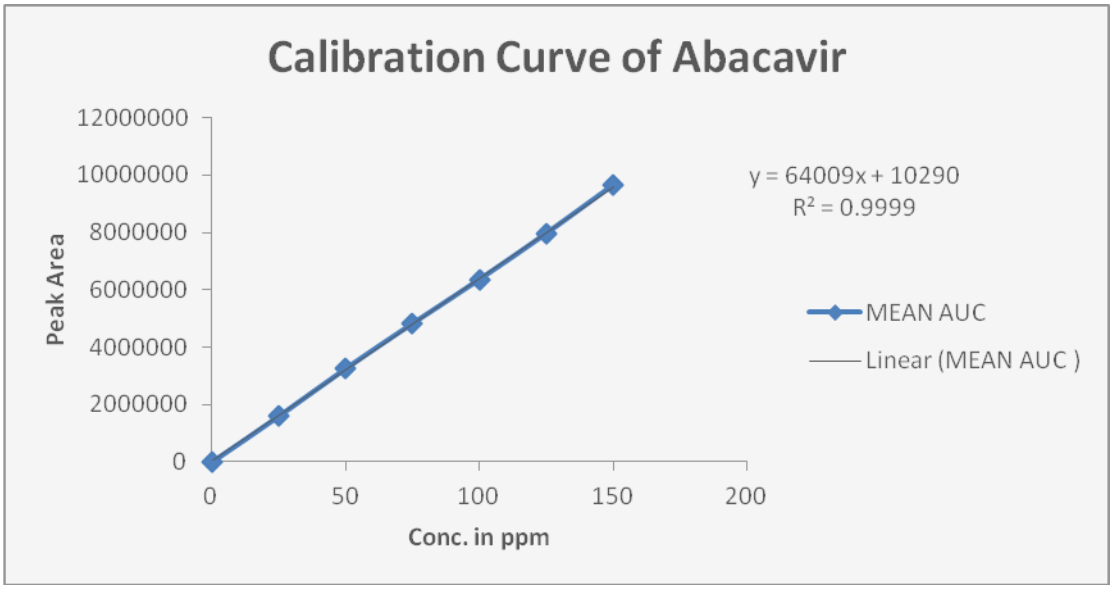

Fig.5. Standard curve for Abacavir

Table-2: Linearity Results for Abacavir

CONC. (µg/ml)	AUC (n=6)
0	0
25	1599571
50	3257873
75	4831264

100	6365428
125	7969987
150	9652641

Lamivudine

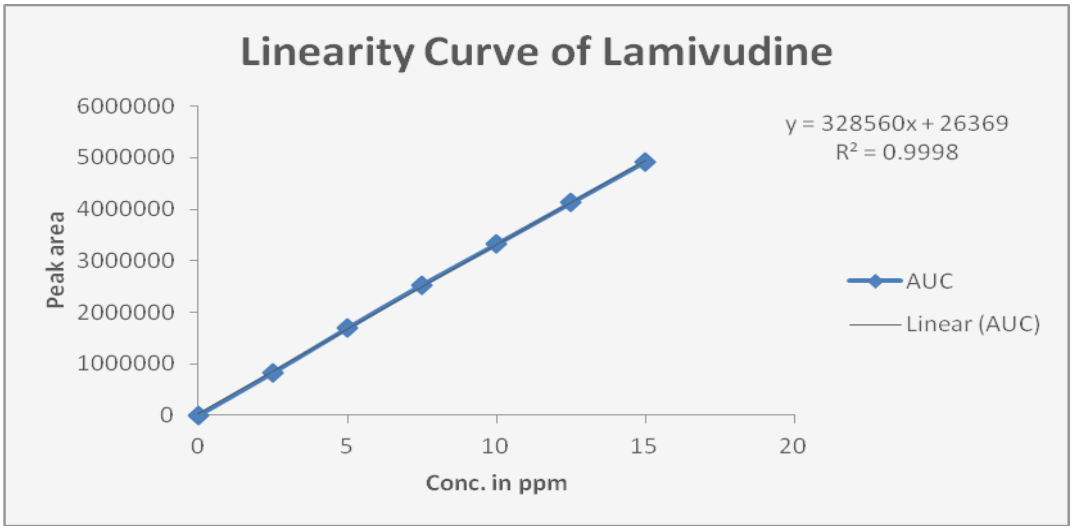

Fig.6. Standard curve for Lamivudine

Table-3: Linearity Results for Lamivudine

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
2.5	831253
5	1694215
7.5	2532451
10	3323412
12.5	4125435

2.6.2. Accuracy:

Table-4: Accuracy Results for Abacavir

Sample ID	Concentration (µg/ml)			% Recovery of Pure drug	Statistical Analysis
	Conc. Found	Conc. Recovered	Peak Area		
S_1 : 80 %	40	39.947	5096978	99.335	Mean= 99.6786%
S_2 : 80 %	40	40.255	5121134	99.806	S.D. = 0.300932
S_3 : 80 %	40	40.292	5125687	99.895	% R.S.D.= 0.301903

S_4 : 100 %	50	49.705	6436424	100.394	Mean= 100.295% S.D. = 0.44484% R.S.D.= 0.443532
S_5 : 100 %	50	50.434	6454876	100.682	
S_6 : 100 %	50	50.858	6398975	99.809	
S_7 : 120 %	60	59.927	7696547	100.066	Mean= 100.0957% S.D. = 0.133986 % R.S.D. = 0.133858
S_8 : 120 %	60	60.414	7710021	100.242	
S_9 : 120 %	60	60.494	7689783	99.979	

Table-5 : Accuracy Results for Lamivudine

Sample ID	Concentration (µg/ml)			% Recovery of Pure drug	Statistical Analysis
	Conc. Found	Conc. Recovered	Peak Area		
S_1 : 80 %	32	32.195	288554	99.737	Mean= 100.208% S.D. = 0.517516 % R.S.D.= 0.516442
S_2 : 80 %	32	31.915	291253	100.762	
S_3 : 80 %	32	32.613	289574	100.125	
S_4 : 100 %	40	40.668	356524	100.48	Mean= 100.5967% S.D. = 1.03055 % R.S.D.= 1.02294
S_5 : 100 %	40	39.738	354528	99.87	
S_6 : 100 %	40	40.310	361121	101.88	
S_7 : 120 %	48	48.181	425361	101.191	Mean= 100.833% S.D. = 0.89769 % R.S.D. = 0.89079
S_8 : 120 %	48	48.085	426534	101.491	
S_9 : 120 %	48	47.813	419897	99.808	

2.6.3. Precision:

2.6.3.1. Repeatability

Table-6: Data showing repeatability analysis for Abacavir & Lamivudine

HPLC Injection Replicates	AUC for Abacavir	AUC for Lamivudine
Replicate – 1	6152684	3378546
Replicate – 2	6212311	3368541
Replicate – 3	6135241	3298786
Replicate – 4	6087958	3352468
Replicate – 5	6125685	3412131
Average	**6142776**	**3362094**
Standard Deviation	**45516.96**	**41582.74**
% RSD	**0.740984**	**1.236811**

2.6.3.2. Intermediate precision:

Table-7: Data for Abacavir analysis

Conc. Of Abacavir (API) (µg/ml)	Observed Conc. of Abacavir (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=3)	% RSD	Mean (n=3)	% RSD
40	40.05	0.89	40.02	0.86
50	49.84	0.35	50.06	0.37
60	59.98	0.19	59.96	0.19

Table- 8: Data for Lamivudine analysis

Conc. Of Lamivudine (API) (µg/ml)	Observed Conc. of Lamivudine (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=3)	% RSD	Mean (n=3)	% RSD
32	31.95	1.08	32.01	0.29
40	40.07	0.57	40.052	0.45
48	48.89	0.75	47.97	0.18

2.6.4. Method Robustness:

Table-9: Result of Method Robustness Test for Abacavir

Change in parameter	% RSD
Flow (0.8 ml/min)	0.72
Flow (1.2 ml/min)	0.65
Wavelength of Detection (251 nm)	0.91
Wavelength of detection (247 nm)	0.63

Table-10 : Result of Method Robustness Test for Lamivudine

Change in parameter	% RSD
Flow (0.8 ml/min)	1.03
Flow (1.2 ml/min)	0.28
Wavelength of Detection (286 nm)	1.04
Wavelength of detection (282 nm)	0.96

2.6.5. LOD & LOQ:lOD: The LOD was found to be 0.35 g/ml and 1.47 g/ml and LOQ was found to be 0.98 g/ml and 2.39 g/ml for Abacavir & Lamivudine respectively which represents that sensitivity of the method is high

2.6.6-Assay of Lercanidipine and Atenolol Tablets

Table-13: Assay of Abacavir & Lamivudine Tablets

Brand name of Tablets	Labelled amount of Drug (mg) Abacavir Lamivudine	Mean (±SD) amount (mg) found by the proposed method (n=6)	Mean (± SD) Assay (n = 6)
ABALAM	600/300	599.68 (±0.06)/299.86 (±0.04)	99.076(±0.48) /99.15(±0.12)

2.6.8 Stability Studies: The various degradation pathways studied are acid hydrolysis, basic hydrolysis, thermal degradation, photolytic degradation and oxidative degradation.

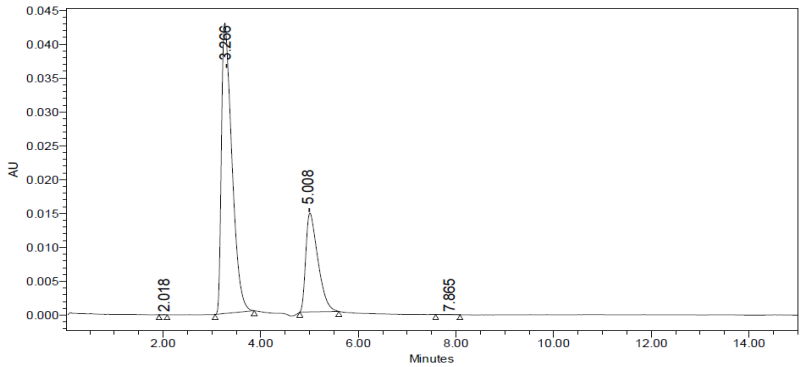

Fig.7. Chromatogram for Acid Degradation

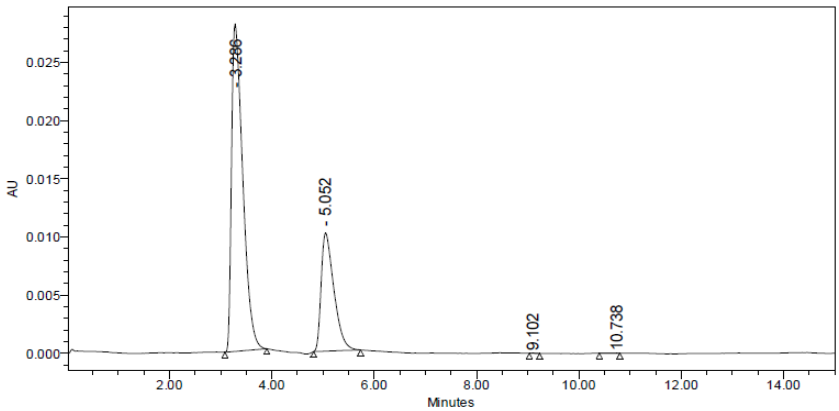

Fig.8. Chromatogram for Basic

Degradation

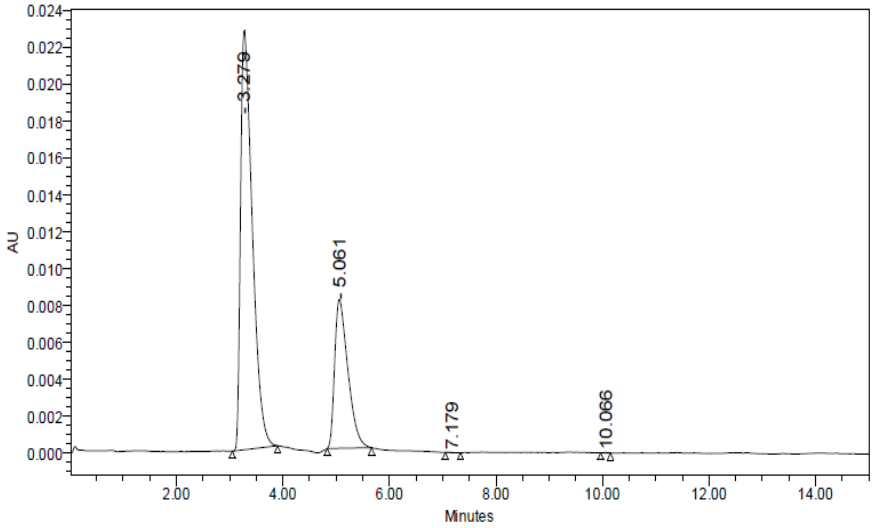

Fig.9. Chromatogram for Thermal Degradation

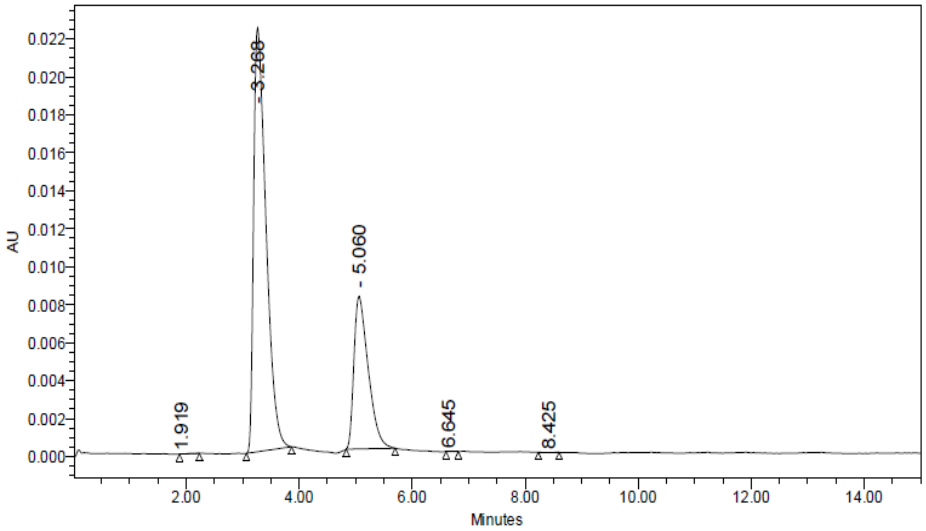

Fig.10.Chromatogram for Photolytic Degradation

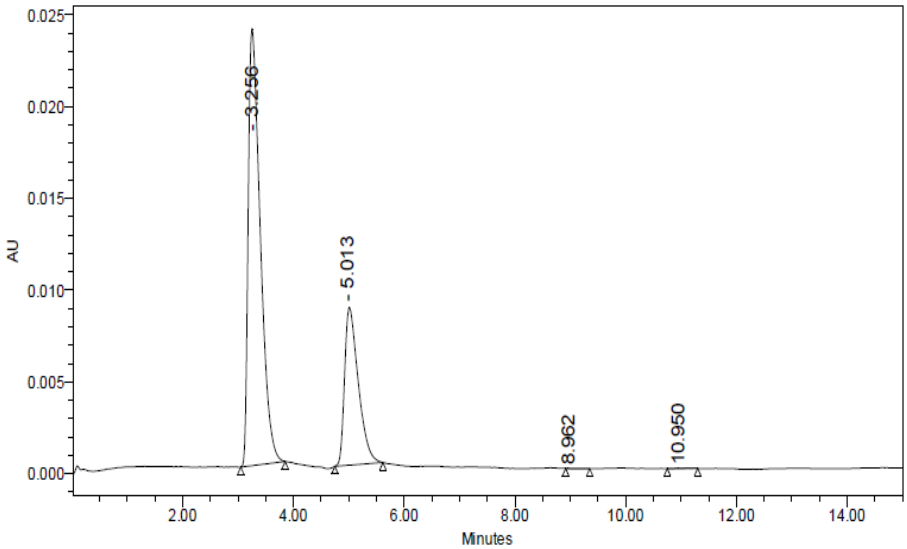

Fig.11.Chromatogram for Oxidation with 3% H_2O_2 Degradation

Table 14-: Results of Force Degradation Studies of Abacavir and Lamivudine API.

Stress condition	Time (hours)	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1N HCl)	24Hrs.	90.65	9.35	100.00
Basic Hydrolysis (0.IN NaOH)	24Hrs.	92.35	7.65	100.00
Thermal Degradation (60 ^{0}C)	24Hrs.	84.24	15.76	100.00
UV (254nm)	24Hrs.	87.21	12.79	100.00
3% Hydrogen peroxide	24Hrs.	74.01	25.91	100.00

III. RESULTS

The optimized chromatographic conditions were Waters ODS (C18) RP Column, 250 mm x 4.6 mm. 5µm as stationary phase and mobile phase was prepared with) ACN:Methanol : Phosphate buffer (0.02M) = 30:35:35 (pH-2.6) and other conditions optimized were: flow rate (1.0 ml/minute), wavelength (284 nm), Run time was maintained at 14 minutes.

In these chromatographic conditions the peak was pure, sharp, symmetric and found a greater number of theoretical plates.

The results obtained in method validation were :

Linearity & Range: Linearity range was found to be 0-60 µg/ml for Abacavir. The correlation coefficient was found to be 0.999, the slope was found to be 11904 and intercept was found to be 12043 for Abacavir.

Linearity range was found to be 0-40 µg/ml for Lamivudine. The correlation coefficient was found to be 0.999, the slope was found to be 15639 and intercept was found to be 2119 for Lamivudine.

Accuracy: The mean recoveries were found to be 100.41, 100.66 and 100.963% for Abacavir and 100.75, 100.59 and 100.05% for Lamivudine. The limit for mean % recovery is 98-102% and as both the values are within the limit, hence it can be said that the proposed method was accurate.

Repeatability: The repeatability study which was conducted on the solution having the concentration of about 50 µg/ml for Abacavir and 40 µg/ml for Lamivudine (n =5) showed a %RSD of 0.870029% for Abacavir and 0.685589% for Lamivudine. It was concluded that the analytical technique showed good repeatability.

LOD & LOQ: The LOD was found to be 0.35 µg/ml and 1.47 µg/ml and LOQ was found to be 0.98 µg/ml and 2.39 µg/ml for Abacavir & Lamivudine respectively which represents that sensitivity of the method is high .

Assay: The assay of Invokamet Tablets containing Abacavir & Lamivudine was found to be 99.076 %. and Lamivudine was found to be 99.15%.

Degradation studies: The results of the stress studies indicated the specificity of the method that has been developed. Abacavir & Lamivudine was more stable in thermal and peroxide stress conditions as compare to other stress conditions.

IV.DISCUSSION

To develop a precise, linear, specific RP-HPLC method for analysis of Abacavir & Lamivudine, different chromatographic conditions were applied & the results observed were compared with the methods available in literatures.

Bimon Routray, et al, . In this present studyA sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of lamivudine and acavir tablet. In case of RP-HPLC various columns are available, but here THERMOSIL C18 150X4.6mm,.5µ or equivalent column was preferred because using this column peak shape, resolution and absorbance were good. A recovery of 101.1% for lamivudine and 99.9%

abacavir was found in assay from tablet formulation.

V.CONCLUSION

A sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of Abacavir & Lamivudine API. Based on peak purity results, obtained from the analysis of samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Abacavir & Lamivudine indicated that the developed method is specific for the estimation of Abacavir & Lamivudine. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

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