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DEVELOPMENT & VALIDATION OF STABILITY INDICATING RP-UPLC METHOD FOR QUANTITATIVE ESTIMATION OF ABACAVIR, LAMIVUDINE AND ZIDOVUDINE IN TABLET DOSAGE FORM

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ABSTRACT

To develop an accurate, simple and reproducible RP-UPLC method for the simultaneous estimation of abacavir, lamivudine and zidovudine in bulk drug and in combined tablet dosage form**.** The separation was achieved by using column Purospher star RP18 Column (50mm X 2.1 mm, 2µ) in mobile phase consisted of pH 6.4 ammonium formate buffer used as mobile phase-A and mobile phase-B used as methanol and acetonitrile in the ratio of 50:50 v/v. Elution mode was gradient, flow rate was 0.3mL/min, column oven temperature 30° C, the injection volume was 1µL, and detection was performed at 272 nm using a photodiode array detector (PDA), Run time 7 minutes. The retention time of abacavir, lamivudine and zidovudine, was noted to be 1.07 minutes 2.8 minutes and 3.8 minutes respectively, indicative of rather shorter analysis time. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible, and consistent.

KEYWORDS: Liquid Chromatography, abacavir, lamivudine and zidovudine, combined dosage forms; Simultaneous estimation, Validation.

1.0 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.[1] 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 prevention in those who have been potentially exposed to HIV. Lamivudine is taken by mouth as a liquid or tablet.

LAM is the (-) enantiomer of a dideoxy analogue of cytidine. LAM was initially developed for the treatment of HIV infection. Although, generally less potent than ZDV in inhibiting HIV-1 and -2 replication in vitro, LAM has very low cellular cytotoxicity. It is rapidly absorbed with a bioavailability of approximately 80%.[2,3]

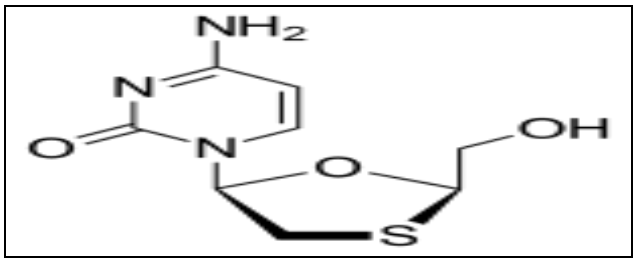

Fig. No. 1.01 structure of Lamivudine.

Abacavir (ABC) is a medication used to prevent and treat HIV/AIDS. Similar to other nucleoside analog reverse-transcriptase inhibitors (NRTIs), abacavir is used together with other HIV medications, and is not recommended by itself. It is taken by mouth as a tablet or solution and may be used in children over the age of three months.

Abacavir is converted by cellular enzymes to the active metabolite, carbovir triphosphate, an analogue of deoxyguanosine–5′ triphosphate. It inhibits the activity of HIV-1 reverse transcriptase both by competing with the natural substrate deoxyguanosine-5′ triphosphate and its incorporation into viral DNA.[2,3] Abacavir is rapidly absorbed following oral administration with a bioavailability of about 80%. It is about 50% bound to plasma proteins. The elimination half-life is about 1.5 h following a single dose.

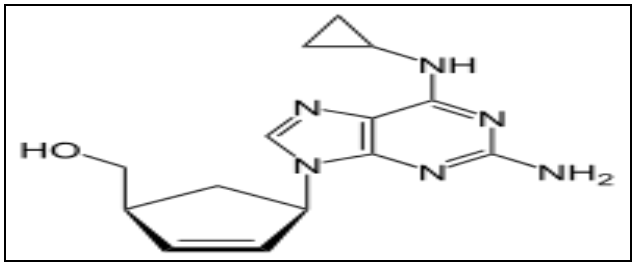

Fig. No. 1.02 structure of Abacavir.

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.[2] It may be used to prevent mother-to-child spread during birth or after a needle stick injury or other potential exposure.

ZDV is a synthetic nucleoside analogue and structurally similar to thymidine. The principal mode of action of ZDV triphosphate is the inhibition of reverse transcriptase via DNA chain termination after incorporation of the nucleoside analogue. ZDV is rapidly absorbed from the gastrointestinal tract with a bioavailability of about 60–70%. It passes the blood-brain barrier.[2,3]

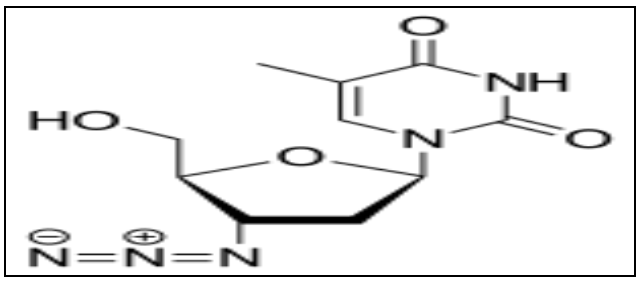

Fig. No. 1.03 structure of Zidovudine.

Literature review reveals some HPLC[4-7], LC-MS/MS[8] and UV spectrophotometric[9-10] and HPTLC[11] methods for their estimation in pharmaceuticals and biological samples. None of the method has been found to estimate the drugs in said combination as per the proposed technique. Hence the present method was developed to quantify Abacavir, Lamivudine and Zidovudine in tablet dosage form by RP-UPLC.

2.0 Experimental

2.1. Chemicals and Reagents

Milli-Q Water, Acetonitrile (HPLC Grade) and ammonium formate (AR Grade) orthophosphoric acid and methanol were obtained from Qualigens Ltd., Mumbai. All other chemical of analytical grade were procured from local sources unless specified. All dilutions were performed in standard class-A, volumetric glassware.

2.2. Instrumentation and Chromatographic Conditions

Instrumentation: Waters 2489 U.V-Visible detector/2695 Separation Module, equipped with Empower 2 software, Bandelin ultrasonic bath, pH Meter (Thermo Orion Model), Analytical Balance (Metller Toledo Model) were use in the present assay.

Preparation of Mobile Phase

Mobile phase A Buffer solution: 0.64 gm of Ammonium formate Salt was dissolved in 1000ml of milli Q water and adjusted the pH to 6.4 with dilute ortho phosphoric acid. Filtered through 0.22µm or a finer porosity membrane filter and degassed.

Mobile phase B

Methanol, Acetonitrile in the ratio of 50:50v/v respectively and degassed.

Preparation of Standard: Weighed accurately and transferred about 75mg of abacavir working standard, 38mg of lamivudine working standard and 75mg of zidovudine working standard into a 25ml volumetric flask. Added 10ml of diluent sonicated to dissolve and diluted to volume with the same. Further dilute 5ml of the above solution to 50ml with diluent and mixed. Filter the solution through 0.22µm nylon filter.

Preparation of sample: Weighed accurately a quantity of the mixed contents of 20 tablets equivalent to about 300mg of abacavir and transferred into a 100ml volumetric flask. Added about 50ml of diluent and sonicated for about 30min and diluted to the volume with diluent. Filter the solution through 0.22µm nylon filter. Further diluted 5ml of the above solution to 50ml with diluent and mixed well.

Chromatographic conditions

Purospher star RP18 Column (50mm X 2.1 mm, 2µ) Column was used for analysis at 30°C column temperature. The mobile phase was pumped through the column at a flow rate of 0.3mL/min. The sample injection volume was 1µL. The photodiode array detector was set to a wavelength of 272nm for the detection and Chromatographic runtime was 7 minutes.

3.0 VALIDATION OF PROPOSED METHOD

Analytical method validation is the process of demonstrating that analytical procedures are suitable for their intended use. More specifically analytical method validation is matter of establishing documented evidence that provides a high degree of assurance that a facility or operation will consistently produce product meeting a predetermined specification.

The following parameters were considered

1. System Suitability
2. Specificity
3. Precision

4. Linearity
5. Accuracy (Recovery)

3.01 System suitability

As per the proposed method chromatographic conditions were set and mobile phase allowed equilibrating with stationary phase. Five replicate injections of working mixed standard solution were injected and the chromatograms were recorded for the drugs.

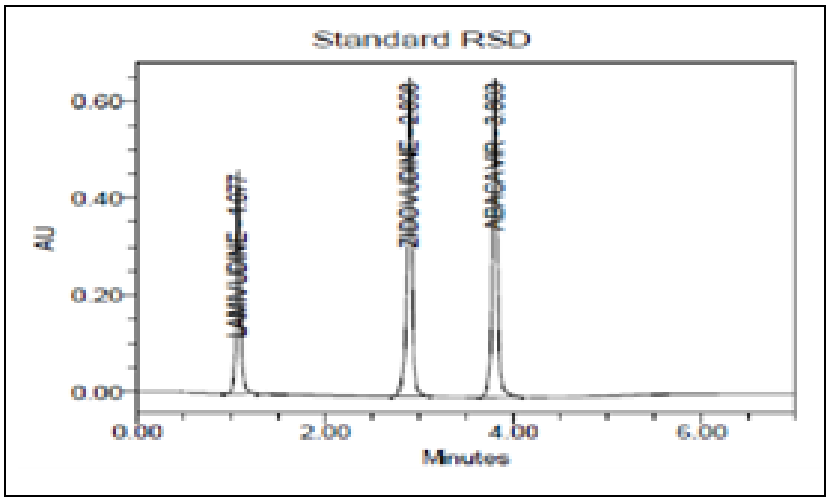

Fig. No. 1.04 typical chromatogram of standard.

Table: 1.01 System suitability data of Abacavir, Lamivudine, Zidovudine.

Parameters	Abacavir	Lamivudine	Zidovudine
	2303844	1433363	2408469
	2300496	1426899	2405245
	2307728	1428595	2411171
	2295450	1425797	2399777
	2298902	1428237	2400539
Mean	2301284	1428578	2405040
%RSD	0.3	0.2	0.3
Theoretical Plates	38389	7434	45521
Resolution		12.37	29.98

3.02. Specificity

Blank interference: Blank was prepared and injected as per test method. It was observed that no blank peaks were interfering with analytical peaks.

Placebo interference: Placebo solutions were prepared in duplicate and injected as per test method. It was observed that no placebo peaks were interfering with analytical peaks.

Impurity interference: All known impurities solution was prepared at about 1% of the test concentration and analyzed as per test method. It was observed that no co elution of the all known impurities peaks with analytical peaks.

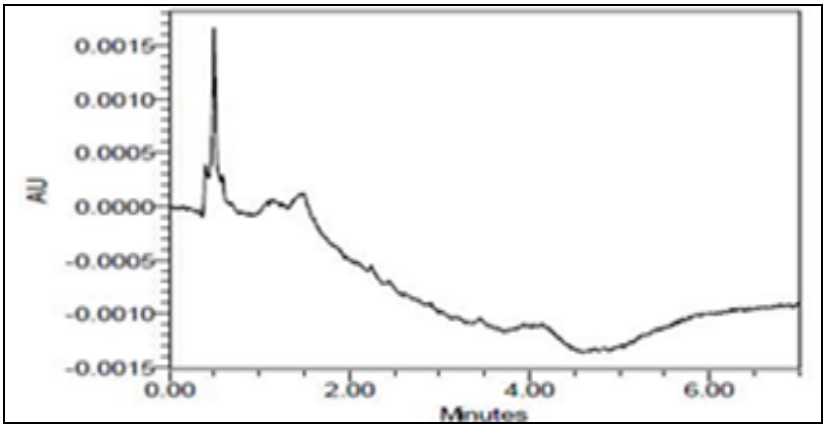

Fig.no:1.05 typical chromatogram of blank.

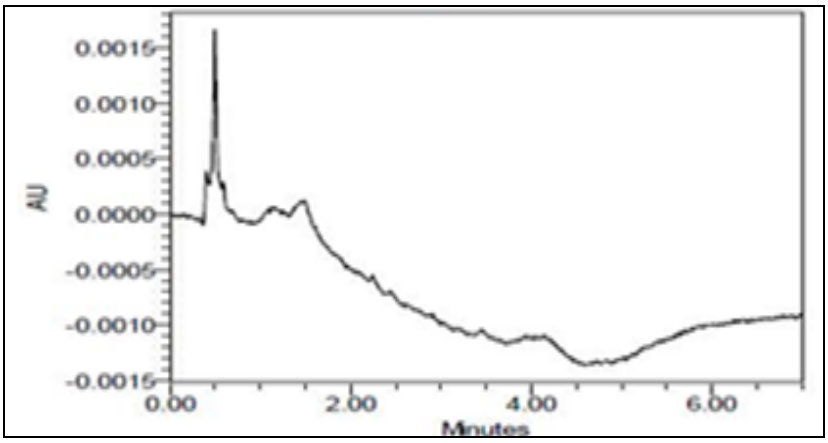

Fig.no: 1.06 typical chromatogram of placebo.

3.03. Linearity: Mixed standard stock solution was diluted with diluent to get the final concentration of standard as 30-250µg/ml .Eight point linearity was determined. Standard solutions of different concentration were injected separately and the chromatograms were recorded. Peak areas were recorded for each injected concentration of drugs and the calibration curves, concentration vs. peak area were constructed for the drugs. Linearity performance parameters are shown in results. The statistical data's for Lamivudine, Abacavir and Zidovudine are shown in results. Dilutions made from the standard stock solution were given in the table.

Table: 1.02 linearity data of lamivudine

Concentration (µg/ml)	Area
33.624	431415
84.059	1089880
134.494	1778971
151.306	2032384
168.112	2224022
184.93	2493842
201.742	2720958
251.117	3389438

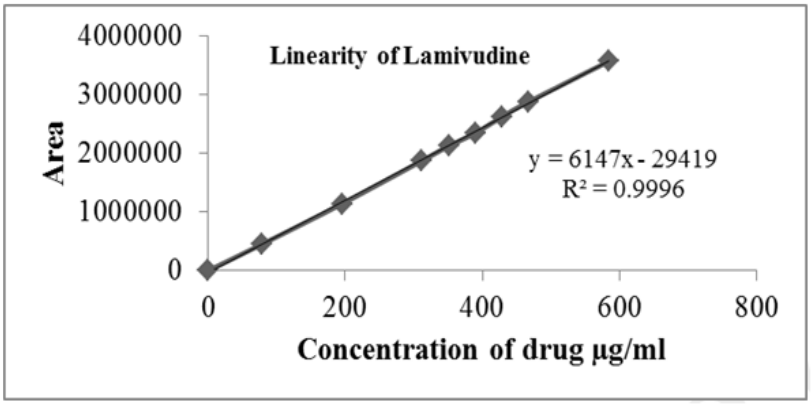

Fig. No: 1.07 Linearity curve of Lamivudine.

Table: 1. 03 linearity data of zidovudine.

Concentration (µg/ml)	Area
67.095	431475
167.738	1088670
268.382	1778641
301.929	2032484
335.477	2224055
369.025	2493632
402.572	2720748
503.215	3389248

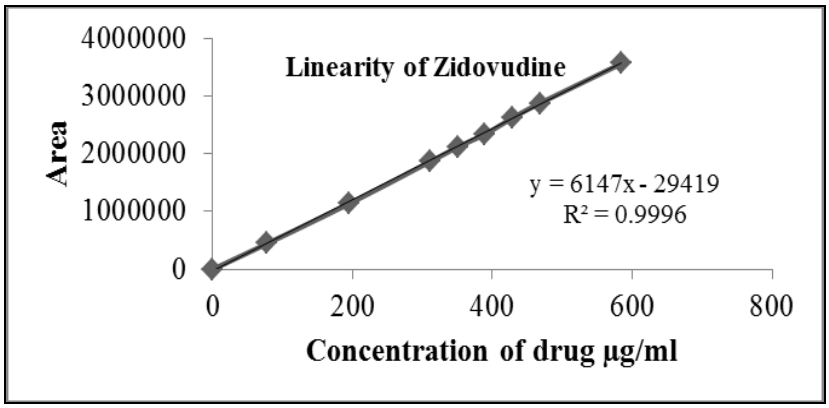

Fig. No: 1.08 Linearity curve of zidovudine.

Table. 1.04 linearity data of Abacavir.

Concentration (µg/ml)	Area
77.94	448015
194.851	1135725
311.761	1871187
350.732	2122387
389.702	2342619
428.672	2622468
467.642	2875634
584.553	3564696

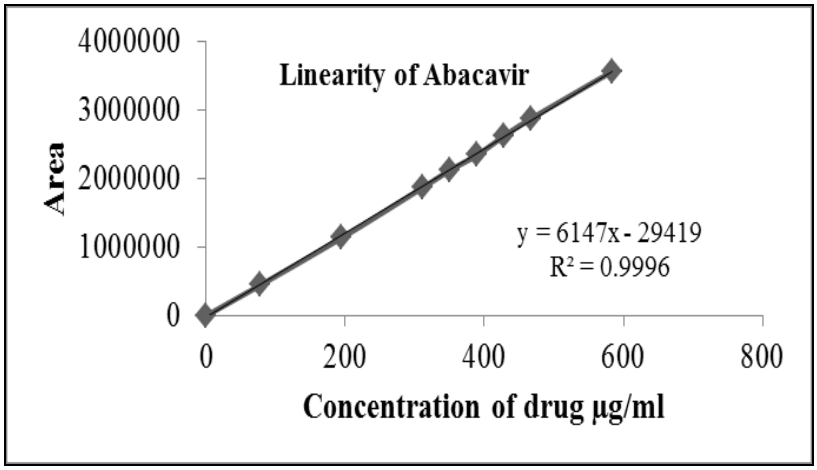

Fig. No: 1.09 Linearity curve of Abacavir.

3.04 Method Precision: Six injections of Sample solution were injected to check the system suitability. Six sets of sample preparation were prepared and each sample was injected in duplicate and chromatograms were recorded.

Table: 1.05 Method precision results.

S. No	Sample Weight (mg)	Area of Lamivudine	% Assay	Area of Zidovudine	% Assay	Area of Abacavir	% Assay
1	1399.96	1289594	95	2264190	101	2409300	100
2	1386.5	1303532	96	2216353	99	2408431	100
3	1393.78	1289192	95	2196594	98	2317339	97
4	1397.41	1329913	98	2242383	100	2285015	95
5	1412.68	1356266	99	2228932	99	2398131	100
6	1402.22	1327005	97	2165714	96	2475476	103
		Mean	97	Mean	99	Mean	99
		%RSD	1.7	%RSD	1.7	%RSD	2.8

Intermediate precision: As per the proposed method, samples were prepared and analyzed by different analyst, different day, different instrument, and different column with different dissolution apparatus. Chromatograms were recorded.

Table: 1.06 Intermediate precision results

S. No	Sample Weight (mg)	Area of Lamivudine	% Assay	Area of Zidovudine	% Assay	Area of Abacavir	% Assay
1	1400.7	1349539	100	2367393	103	2251695	102
2	1417.4	1377564	102	2387108	104	2252941	102
3	1406.9	1321488	98	2339061	102	2259294	102
4	1410.7	1386348	102	2388523	104	2269986	103
5	1410.2	1371562	101	2381027	104	2311658	105
6	1412.8	1386005	102	2430110	106	2188165	99
		Mean	101	Mean	104	Mean	102
		%RSD	1.7	%RSD	1.3	%RSD	1.8

3.05 Accuracy: A series of solutions were prepared by spiking the placebo and API in the range of about 50% to 150% of test concentration in triplicate and injected into HPLC system and analyzed as per the test method. Calculated individual % recovery and mean % recovery at each level and the results were found to be within the acceptable limits.

Table: 1.07 Accuracy results for Lamivudine.

S. No.	% Spike level	Amount added (mg)	Amount recovered (mg)	% Recovery	% Mean recovery
1	50	74.68	73.49	98.4	98.6
2		74.49	73.70	98.9	
3		74.85	73.73	98.5	
1	100	149.27	149.01	99.8	99.8
2		149.12	148.80	99.8	
3		149.33	148.82	99.7	
1	150	225.45	225.38	100.0	100.1
2		225.66	225.89	100.1	
3		225.33	225.79	100.2	

Table: 1.08 Accuracy results for Zidovudine.

S. No.	% Spike level	Amount added (mg)	Amount recovered (mg)	% Recovery	% Mean recovery
1	50	149.53	150.64	100.7	100.8
2		149.57	150.31	100.5	
3		149.03	150.70	101.1	
1	100	298.59	300.78	100.7	100.7
2		298.29	300.46	100.7	
3		298.75	300.59	100.6	
1	150	450.53	450.80	100.1	100.1
2		450.74	450.93	100.0	
3		450.05	450.20	100.0	

Table: 1.09: Accuracy data of Abacavir.

S. No.	% Spike level	Amount added (mg)	Amount recovered (mg)	% Recovery	% Mean recovery
1	50	149.37	148.37	99.3	99.1
2		149.13	147.73	99.1	
3		149.33	147.64	98.9	
1	100	298.53	296.97	99.5	99.6
2		298.28	298.08	99.9	
3		298.74	297.24	99.5	
1	150	450.31	449.54	99.8	100.0
2		450.05	450.15	100.0	
3		450.45	450.90	100.1	

4.0. CONCLUSION

An attempt has been made to develop the method using UPLC method for simultaneous estimation of Lamivudine, Abacavir, and Zidovudine in combined dosage form. As the literature survey revealed that few methods are available for simultaneous estimation of Lamivudine, Abacavir and Zidovudine in combined dosage form but there is a need of a simple, economical and proper method for estimation of above combination in combined dosage form. Waters Alliance UPLC-empower software with PDA detector with Purospher star RP18(50×2.1×2µ) column with an injection volume of 1µl is injected and eluted with the mobile phase of 0.01 M Ammonium formate buffer : ACA, MeOH (50:50), pH-6.4 which is pumped at a flow rate of 0.3ml/min and detected by UV detector at 272 nm. The peaks of Lamivudine, Zidovudine and Abacavir are found well separated at 1.07, 2.8, and 3.7respectively. The developed chromatographic method for the determination of test procedures of %Assay for Lamivudine, Zidovudine and Abacavir in bulk drug and tablets were simple, rapid, accurate, precise, specific, robust and economical. The mobile phase is easy to prepare. This method is also having an advantage than reported method of good resolution and with retention time. Thus the method is not time consuming and can be used in laboratories for the routine analysis of combination drugs. Since the system suitability studies of UPLC method used for simultaneous estimation of active ingredients (Lamivudine, Abacavir& Zidovudine) in tablet formulation as well as its validation studies have shown satisfactory, accurate and reproducible results (without any interference of excipients) as well, it is deduced that the simple and short proposed method be most useful for analysis purpose.

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