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Simultaneous RP-HPLC determination of abacavir, lamivudine and dolutegravir in bulk API dosage forms

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ABSTRACT

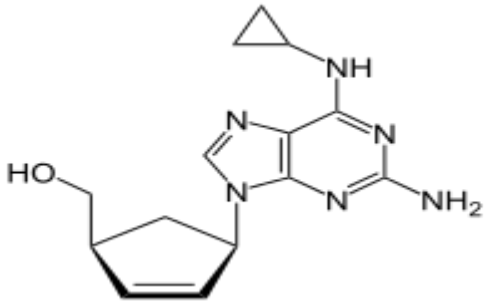
The RP-HPLC method was developed and validated for the determination of a combination of Abacavir, Lamivudine and Dolutegravir in bulk API forms. The chromatography was carried out on Agilent C_{18} column (100mm x 4.6mm, 3.5µ) using a mobile phase of Ammonium formate and methanol in the ratio of 40:60, at a flow rate of 0.8ml/minute. The analytes were monitored at 262nm using a PDA detector. The retention time of Abacavir, Lamivudine and Dolutegravir were observed at 1.73, 1.30 and 4.32 minutes respectively. The developed method was found to be linear in the concentration range of 5µg-50µg having r^2 value of 0.999, 0.999 and 0.998 for Abacavir, Lamivudine and Dolutegravir respectively. The method has been validated according to ICH guidelines.

KEYWORDS: RP-HPLC, Abacavir, Lamivudine, Dolutegravir, combination

INTRODUCTION

ABACAVIR

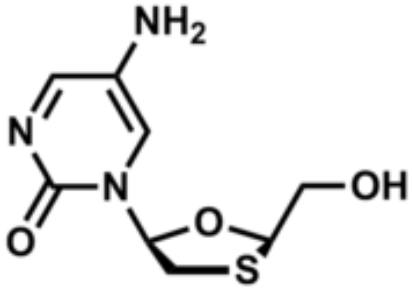
Abacavir is chemically (1S,4R)-4[2-amino-6(cyclo propylamic)-9H-purin-9yl]cyclopent-2-en-1-yl} ethanol. It has a molecular formula of $C_{14}H_{18}N_6O$, and has a molecular weight of 286.332g/mol. Abacavir is an anti-retroviral drug used in the treatment of HIV-AIDS. It is of nucleoside analogue reverse transcriptase inhibitor. Intracellularly, Abacavir is converted by cellular enzyme to the active metabolite carbovir triphosphate, an analogue of deoxygeanosine-5^1-triphosphate. Carbovir triphosphate inhibits the activity of HIV-1 reverse transcriptase both by competing with the natural substrate dGTP and by its incorporation into viral DNA.



Structure of Abacavir

LAMIVUDINE

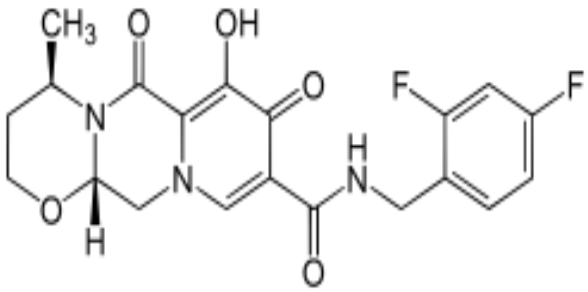
Chemically it is "4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5yl]-1,2-dihydropyrimidin-2-one". It has a molecular formula of $C_8H_{11}N_3O_3S$ and has a molecular weight of 229.26g/mol. Lamivudine is an analogue of cytidine. It can inhibit both types (1 and 2) of HIV reverse transcriptase and also the reverse transcriptase of Hepatitis B virus. It is phosphylated to active metabolites that compete for incorporation into the viral DNA.



Structure of Lamivudine

DOLUTEGRAVIR

Chemically it is "(4R, 12aS)-N-(2,4-diflurobenzyl)-7hydroxy 4methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2H-pyrido[1^1,2^1,4,5]pyrazinol(2,1-b) [1,3]oxazine-9-carboxamide. It has a chemical formula of $C_{20}H_{19}F_2N_3O_5$ and a molecular weight of 419.38g/mol. Dolutegravir is an integrase inhibitor, HIV-1-antiviral agent. It inhibits HIV integrase by binding to the active site and blocking the strand transfer step of HIV replication cycle and will result in a inhibition of viral activity.



Structure of Dolutegravir

MATERIALS AND METHODS

INSTRUMENTATION

Agilent technologies HPLC system was used, which was operated by open lab software and monitored by UV and DAD detector for both method development and validation.

MATERIALS

Methanol (HPLC grade) obtained from Loba chemie Pvt. ltd. Ammonium formate (qualigens) obtained from Fisher scientific India Pvt. ltd. Milli-Q water was used throughout the experiment.

PREPARATION OF STANDARD SOLUTIONS

The standard stock solution were prepared by taking accurately 100mg of each Abacavir, Lamivudine and Dolutegravir in a 100ml volumetric flask, and was made upto volume using diluent of methanol 50%. The Sample solutions were prepared by taking 1.2ml, 0.6ml and 0.1 ml of Abacavir, Lamivudine and Dolutegravir from the stock solutions to a 10ml volumetric flask and was made upto the volume using methanol 50%.

RESULTS AND DISCUSSIONS

CHROMATOGRAPHIC CONDITIONS

To develop the proposed RP-HPLC method, a number of ratios involving the mobile phase, pH, flow rate parameters were altered and trials were performed. The peak shape, symmetry and resolution factors were found to be good with a flow of 0.8ml/min. The mobile phase used was ammonium formate buffer of pH 4.0(adjusted using formic acid) and methanol in the ratio of 40:60. Agilent C_{18} column (100mm x 4.6mm, 3.5µ) was used. The analytes were monitored using DAD at a wavelength of 262nm. The run time of the proposed method is 6minutes. The retention time of Abacavir, Lamivudine and Dolutegravir were found to be at 1.73, 1.30 and 4.32 minutes.

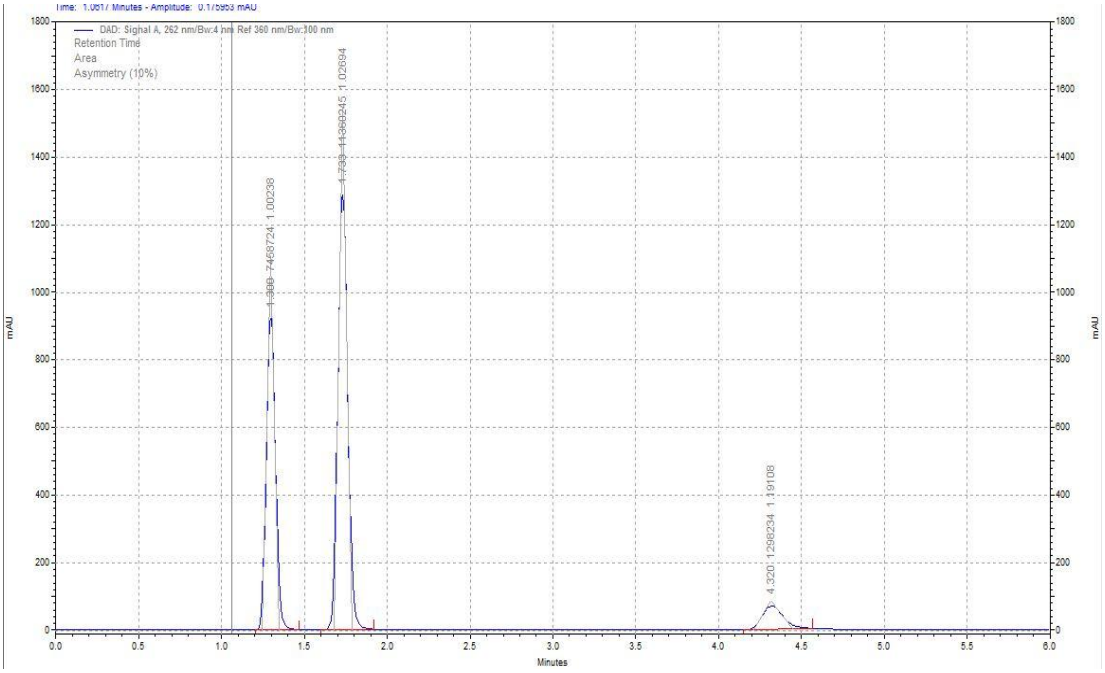


Figure-1: Standard chromatogram of Abacavir, Lamivudine and Dolutegravir

METHOD VALIDATION

The optimized RP-HPLC method was validated according to ICH guidelines.

LINEARITY

The linearity of Abacavir, Lamivudine and Dolutegravir were found by preparing standard solutions at six different concentrations ranging from 5µg-50µg/ml. Each concentration was injected for five times and the mean value of the peak area was used for the construction of the calibration curve. As shown in figure:2, figure:3 and figure:4.

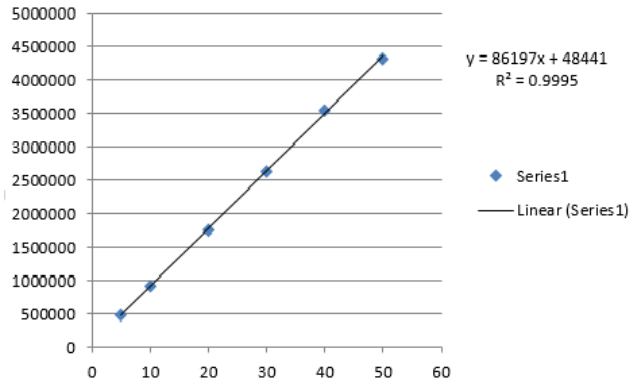


Figure-2: linearity of Abacavir

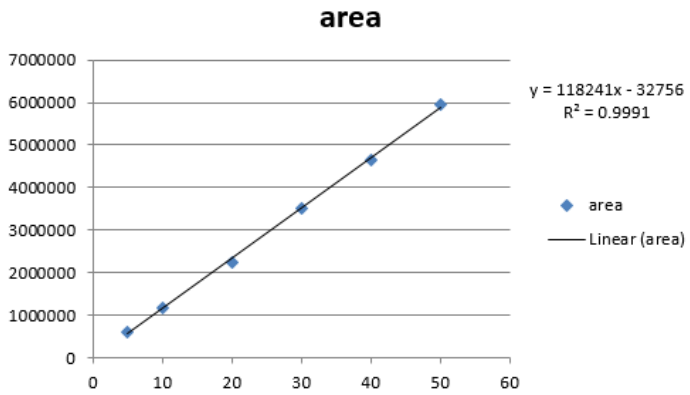


Figure-3: linearity of Lamivudine

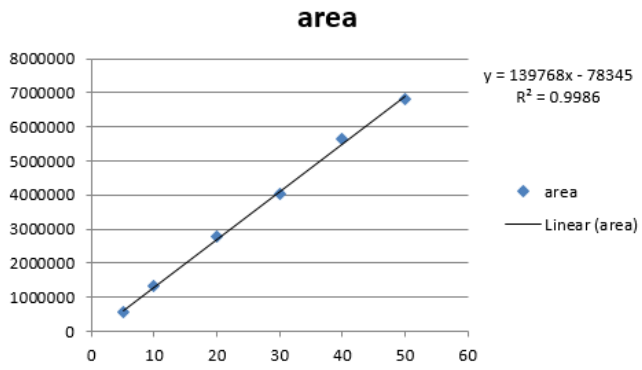


Figure-4: linearity of Dolutegravir

PRECISION

System precision was carried out by injecting standard solution preparations six times into the chromatographic system and the %RSD values were calculated for retention time and peak area for all the three drugs, which were found to be within limits. Results were shown in Table: 1.

Table-1: Intraday and Inter day precision

	Parameters	**Abacavir**	**Lamivudine**	**Dolutegravir**
Intra day precison	%RSD retention time	0.00	0.31	0.19
	%RSD peak area	1.12	1.19	0.91
Inter day precision	%RSD retention time	0.00	0.30	0.18
	%RSD peak area	1.14	1.15	0.20

ACCURACY

The accuracy of the assay method was calculated for Abacavir, Lamivudine and Dolutegravir by recovery studies at three different concentrations i.e. 50%, 100% and 150% levels by using standard addition method and each concentration was injected three times. The accuracy of an analytical method should be established across its range. Results obtained are given in table: 2, table: 3, table: 4.

Table -2: Accuracy values of Abacavir

S.No	%Level	Total Amount	Amount Found	%Recovery	Mean Recovery
1	50%	60	59.03	98.39	Mean-99.82
2	50%	60	59.99	99.98	SD-0.3837
3	50%	60	60.07	100.11	%RSD-0.38
4	100%	120	118.64	98.87	Mean-99.33
5	100%	120	119.94	99.95	SD-0.5574
6	100%	120	119.01	99.17	%RSD-0.56
7	150%	180	180.42	100.23	Mean-99.49
8	150%	180	179.19	99.55	SD-0.7614
9	150%	180	177.68	98.71	%RSD-0.77

Table-3: Accuracy values of Lamivudine

S.No	%Level	Total Amount	Amount Found	%Recovery	Mean Recovery
1	50%	30	29.74	99.13	Mean-99.07
2	50%	30	29.68	98.94	SD-0.1159
3	50%	30	29.74	99.15	%RSD-0.12
4	100%	60	59.70	99.54	Mean-99.55
5	100%	60	59.76	99.51	SD-0.0458
6	100%	60	59.70	99.60	%RSD-0.05
7	150%	90	90.37	100.44	Mean-99.55
8	150%	90	89.16	99.07	SD-0.768
9	150%	90	89.23	99.15	%RSD-0.77

Table-4: Accuracy values of Dolutegravir

S.No	%Level	Total Amount	Amount Found	%Recovery	Mean Recovery
1	50%	5	4.92	98.57	Mean-98.59
2	50%	5	4.93	98.63	SD-0.0305
3	50%	5	4.92	98.59	%RSD-0.03
4	100%	10	9.89	98.93	Mean-99.39
5	100%	10	9.97	99.73	SD-0.4147
6	100%	10	9.95	99.52	%RSD-0.42
7	150%	15	15.04	100.27	Mean-99.52
8	150%	15	14.95	99.72	SD-0.856
9	150%	15	14.78	98.57	%RSD-0.86

SYSTEM SUITABILITY

System suitability was carried out by injecting 20 µl of standard solution in six replicates. The system suitability parameters were evaluated for tailing factor, theoretical plates, retention time and area. %RSD for peak areas was calculated (%RSD NMT 2) were found to be within the limits. The obtained values are reported in table 5.

Table-5: System suitability parameters

Parameters	Abacavir	Lamivudine	Dolutegravir
Retention time	1.73	1.30	4.32
Tailing factor	1.1	1.1	1.6
Theoretical plates	4144	2885	5430
%RSD of peak area	1.06	1.12	1.33

ROBUSTNESS

Robustness of the proposed method was determined by analyzing the standard solution by changing the physical parameters like flow rate, mobile phase composition, pH of the buffer. The results obtained are shown in the table-6, table-7 and table-8.

Table -6: Robustness values of Abacavir

PARAMETER	**CHANGE LEVEL**	**Abacavir**			
		RT (min)	**Peak Area**	**Tailing factor**	**USP Plate Count**
Flow Rate (±0.1ml/min)	0.7ml/min	2.03	11497512	1.01	4189
	0.9ml/min	1.58	8987627	1.02	3421
Mobile phase organic composition	39:61	1.75	10300052	1.02	3775
	41:59	1.82	10227890	1.01	3641
Buffer pH	P^H 3.9	1.75	11489733	1.00	3868
	P^H 4.1	1.72	11548397	1.01	3908

Table-7: Robustness values of Lamivudine

PARAMETER	CHANGE LEVEL	Lamivudine			
		RT (min)	Peak Area	Tailing factor	USP Plate Count
Flow Rate (±0.1ml/min)	0.7ml/min	1.49	7772309	0.98	2711
	0.9ml/min	1.15	6232239	1.02	2205
Mobile phase organic composition	39:61	1.30	6880781	1.00	2200
	41:59	1.31	6967024	1.03	2350
Buffer pH	P^H 3.9	1.30	7566893	1.00	2579
	P^H 4.1	1.29	7482434	1.10	2493

Table-8: Robustness values of Dolutegravir

PARAMETER	CHANGE LEVEL	Dolutegravir			
		RT (min)	Peak Area	Tailing factor	USP Plate Count
Flow Rate (±0.1ml/min)	0.7ml/min	5.07	1629078	1.11	6003
	0.9ml/min	3.90	1432480	1.10	4379
Mobile phase organic composition	39:61	4.09	1521620	1.11	5183
	41:59	4.74	1465680	1.08	5052
Buffer pH	P^H 3.9	4.32	1632279	1.04	5140
	P^H 4.1	4.31	1622552	1.04	5161

CONCLUSION

An accurate, precise and suitable method was developed and validated for the combination of Abacavir, Lamivudine and Dolutegravir in bulk API dosage forms. The proposed method is rapid method and can be applied to routine quality analysis.

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