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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ABACAVIR, LAMIVUDINE & DOLUTEGRAVIR BY RP-HPLC METHOD

Ponnamalla Jasmine Carey*, Dr. Devanaboyina Narendra and Gadi Vijaya Lakshmi

Department of Pharmaceutical Analysis & Quality Assurance VJ'S College of Pharmacy, Diwancheruvu, Rajamahendravaram, Andhra Pradesh-533296.

***Corresponding Author: Ponnamalla Jasmine Carey**
Department of Pharmaceutical Analysis & Quality Assurance VJ'S College of Pharmacy, Diwancheruvu, Rajamahendravaram, Andhra Pradesh-533296.

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the abacavir, lamivudine and dolutegravir tablet dosage form. Chromatogram was run through STD Agilent C18 150 x 4.6 mm, 5µ. Mobile phase containing Water: Acetonitrile taken in the ratio 60:40 was pumped through column at a flow rate of 0.8 ml/min. Buffer used in this method was milli-Q Water. Temperature was maintained at 30°C. Optimized wavelength selected was 284 nm. Retention time of Abacavir/Lamivudine/ Dolutegravir were found to be 2.233 min and 2.700,3.426. %RSD of the Abacavir/Lamivudine/ Dolutegravir were and found to be 0.4,0.5 and 0.3 respectively. %Recovery was obtained as 99.89%, 100.0.4%and 100.35% for abacavir/Lamivudine/ dolutegravir respectively. LOD, LOQ values obtained from regression equations of Abacavir/Lamivudine/ dolutegravir were 0.04, 0.11,0.08 and 0.13, 0.33,0.24respectively. Regression equation of Abacavir is y = 37926x + 8085.1, y = 38697x + 13271 of Lamivudine and dolutegravir is y= 4326.9x+194.59. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular quality control test in Industries.

KEYWORDS: Abacavir, Lamivudine and Dolutegravir, RP-HPLC.

INTRODUCTION

Chemically Abacavir (ABC) is a carbocyclic synthetic nucleoside analogue and an antiviral agent. Intracellularly, abacavir is converted by cellular enzymes to the active metabolite carbovir triphosphate, 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 dGTP and by its incorporation into viral DNA. Viral DNA growth is terminated because the incorporated nucleotide lacks a 3'-OH group, which is needed to form the 5′ to 3′ phosphodiester linkage essential for DNA chain elongation. Structure of the ABC was shown in figure 1 (A).[1]

Chemically Lamivudine (LMD) is a synthetic nucleoside analogue and is phosphorylated intracellularly to its active 5'-triphosphate metabolite, lamivudine triphosphate (L-TP). This nucleoside analogue is incorporated into viral DNA by HIV reverse transcriptase and HBV polymerase, resulting in DNA chain termination. Structure of the LMD was shown in figure 1 (B).[2]

Chemically Dolutegravir (DLT) is an HIV-1 antiviral agent. It inhibits HIV integrase by binding to the active site and blocking the strand transfer step of retroviral DNA integration in the host cell. The strand transfer step is essential in the HIV replication cycle and results in the inhibition of viral activity. Dolutegravir has a mean EC50 value of 0.5 nM (0.21 ng/mL) to 2.1 nM (0.85 ng/mL) in peripheral blood mononuclear cells (PBMCs) and MT-4 cells. Structure of the DLT was shown in figure 1 (C).[3]

Literature survey reveals there are several methods to estimated these drugs in single or in combination of two or three drugs [5-9]. But there is only very few HPLC methods are available for simultaneous estimation of ABC, LMD and DLT, so the scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte to be more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation processes.

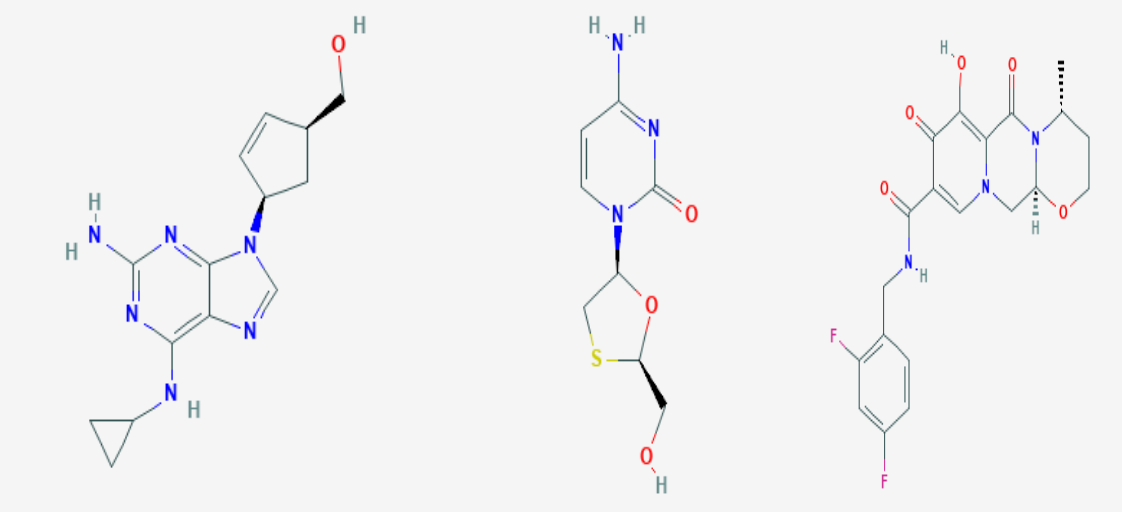

Figure 1: Structure of (A) Abacavir (B) Lamivudine (C) Dolutegravir

MATERIALS AND METHODS

Reagents and Chemicals: Abacavir, Lamivudine & Dolutegravir pure drugs (API), Combination Abacavir, Lamivudine & Dolutegravir, Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

Instrumentation: HPLC (waters 2695) system with Empower-2 software and 2996 module photo diode array detector equipped with a quaternary solvent delivery pump, automatic sampler unit, Agilent C18 (4.6 x 150mm, 5µm). As part of experimentation, additional equipment such as sonicator (ultrasonic cleaner power sonic 420), pH meter, vacuum oven (wadegati), water bath and other glassware were used for the present investigation.

Chromatographic conditions: The Agilent C18 (4.6 x 150mm, 5µm) column was used for analytical separation. Potassium dihydrogen ortho phosphate and one drop of triethyl amine in every 100ml of Acetonitrile and water was taken in the ratio of (60:40%v/v) mobile phase for the investigation with a flow rate of a 0.8ml/min. The temperature was maintained at 30^0C. The injection volume was 10µl and the UV detection was achieved at 284nm.

Preparation of potassium dihydrogen ortho phosphate buffer (pH:3.0): Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 3.45 with dil. Orthophosphoric acid solution.

Preparation of mobile phase

Buffer: Water - in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water

Preparation of mixture Standard stock solution: Accurately Weighed and transferred 30mg, 15mg & 25mg of abacavir, lamivudine, dolutegravir working Standards into a 50ml clean dry volumetric flask, add 25ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solution (600µg/ml of abacavir and 300µg/ml lamivudine, 500 µg/ml of dolutegravir).

Preparation of Sample (Tablet) stock solutions: 20 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 500ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (600µg/ml of abacavir and 300µg/ml lamivudine, 500 µg/ml of dolutegravir).

Optimized chromatographic conditions

Column Used : Agilent C18 (4.6 x 150mm, 5µm)
Mobile phase : Acetonitrile: Water (60:40v/v)
Flow rate : 0.8ml/min
Wavelength : 284.0 nm
Temperature : 30◦C
Injection Volume: 10.0µl

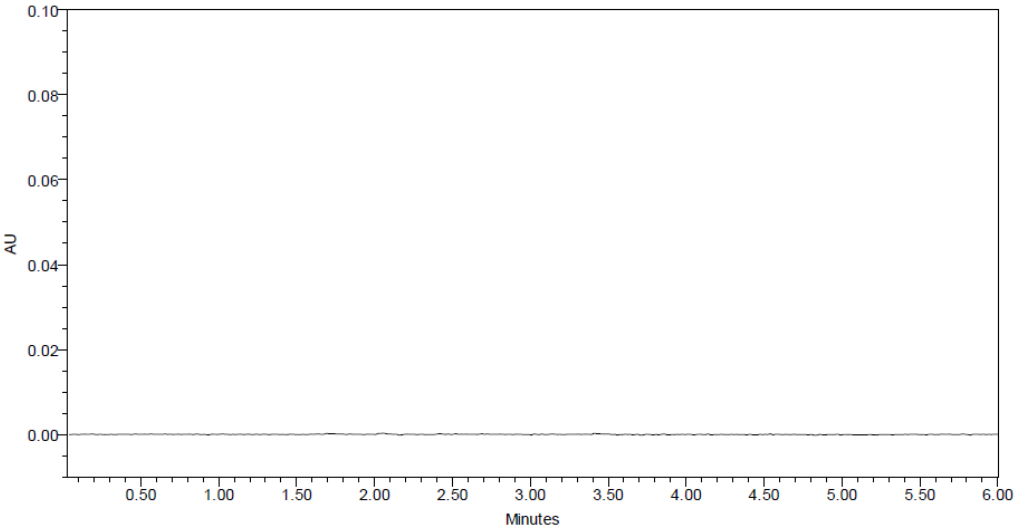

Figure 2: Blank chromatogram.

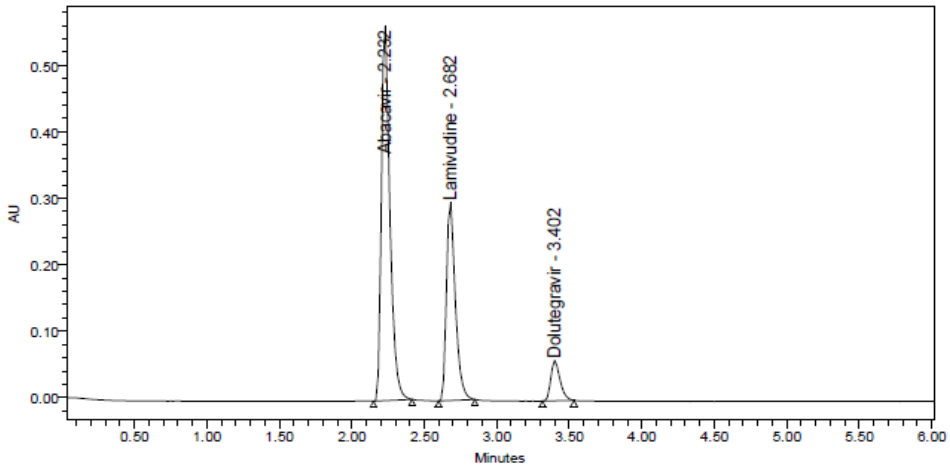

Figure 3: Chromatogram of standard mixture of ABC, LMD & DLT.

	Peak Name	RT	Area	USP Tailing	USP Resolution	USP Plate Count
1	Abacavir	2.232	2292575	1.37	6	6301
2	Lamivudine	2.682	1258304	1.37	4.1	8537
3	Dolutegravir	3.402	258928	1.31	6	13265

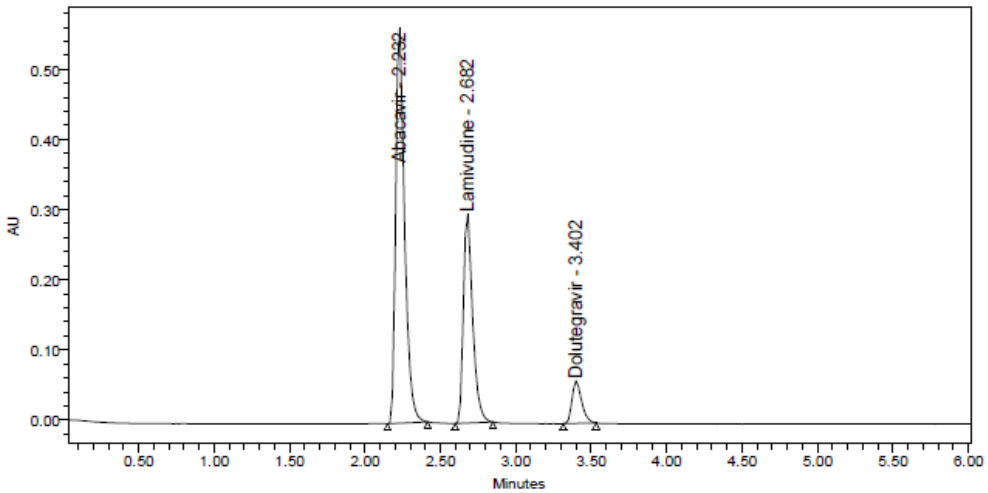

Figure 4: Chromatogram of sample mixture of ABC, LMD & DLT.

Table 1: Linearity table for ABC, LMD & DLT.

Abacavir		Lamivudine		Dolutegravir	
Conc (µg/mL)	Peak area	Conc (µg/mL	Peak area	Conc (µg/mL)	Peak area
0	0	0	0	0	0
15	576372	7.5	329900	12.5	55103
30	1146707	15	595998	25	108119
45	1719550	22.5	866836	37.5	160357
60	2295508	30	1173062	50	221315
75	2862839	37.5	1456450	62.5	265402
90	3402461	45	1765379	75	326869

Table 2: System precision table of ABC, LMD & DLT.

S. No	Area of Abacavir	Area of Lamivudine	Area of Dolutegravir
1.	2286557	1260761	259614
2.	2271244	1261048	253771
3.	2253882	1248978	260347
4.	2299784	1237957	259784
5.	2322035	1274424	259183
6.	2315931	1266653	260866
Mean	2292575	1258304	258928
S.D	26259.1	12993.1	2593.5
%RSD	1.1	1.0	1.0

Table 3: degradation data of ABC.

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	20.39	0.127	0.287
2	Alkali	20.08	0.166	0.285
3	Oxidation	21.48	0.373	0.422
4	Thermal	23.35	0.175	0.287
5	UV	21.50	0.186	0.285
6	Water	19.77	0.190	0.286

Table 4: Degradation data of LMD.

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	18.75	0.139	0.332
2	Alkali	20.17	0.138	0.335
3	Oxidation	18.29	0.152	0.323
4	Thermal	25.30	0.149	0.322
5	UV	23.74	0.139	0.314
6	Water	25.25	0.139	0.312

Table 5: Degradation data of DLT.

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	28.93	0.239	0.321
2	Alkali	21.51	0.238	0.305
3	Oxidation	22.24	0.229	0.508
4	Thermal	19.25	0.290	0.521
5	UV	26.48	0.189	0.304
6	Water	27.30	0.261	0.482

Table 6: Summary of validation data of RTN, OMB & PRP.

Parameters		Abacavir	Lamivudine	Dolutegravir	LIMIT
Linearity Range (µg/ml)		15-90 µg/ml	7.5-45 µg/ml	12.5-75µg/ml	R< 1
Regression coefficient		0.999	0.999	0.999	
Slope(m)		37926	38697	4326.9	
Intercept(c)		8085.1	13271	194.59	
Regression equation (Y=mx+c)		y = 37926x + 8085.1	Y=38697 x+13271	y = 4326.9x + 194.59	
Assay (% mean assay)		99.44%	99.97%	100.26%	90-110%
Specificity		Specific	Specific	Specific	No interference of any peak
System precision %RSD		1.1	1.0	1.0	NMT 2.0%
Method precision %RSD		0.4	0.5	0.3	NMT 2.0%
Accuracy %recovery		99.89%	100.04%	100.35%	98-102%
LOD		0.04	0.11	0.08	NMT 3
LOQ		0.13	0.33	0.24	NMT 10
Robustness	**FM**	0.5	0.6	1.6	%RSD NMT 2.0
	FP	0.7	0.8	0.6	
	MM	1.3	1.2	0.5	
	MP	0.5	0.3	0.7	
	TM	0.1	0.3	0.8	
	TP	0.4	0.3	1.0	

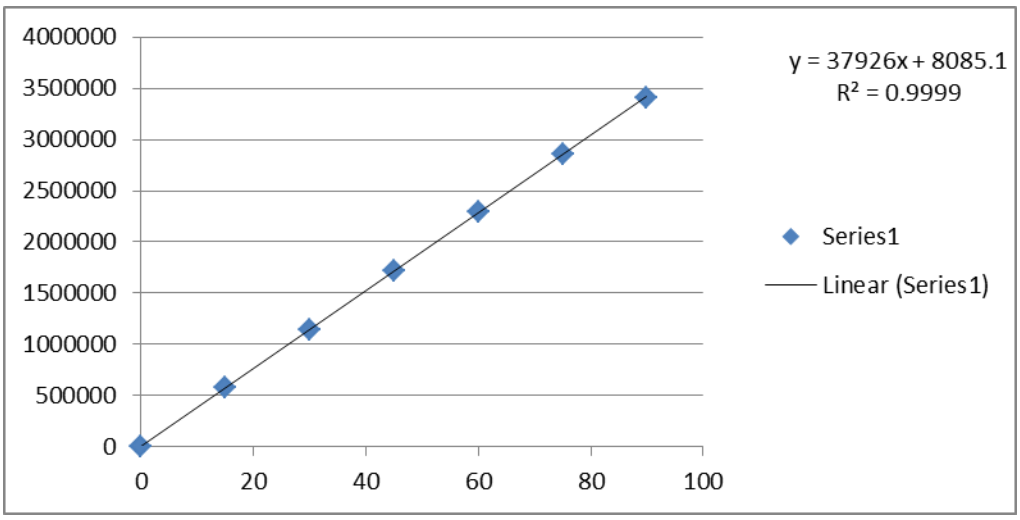

Fig. 7: Linearity curve of Abacavir.

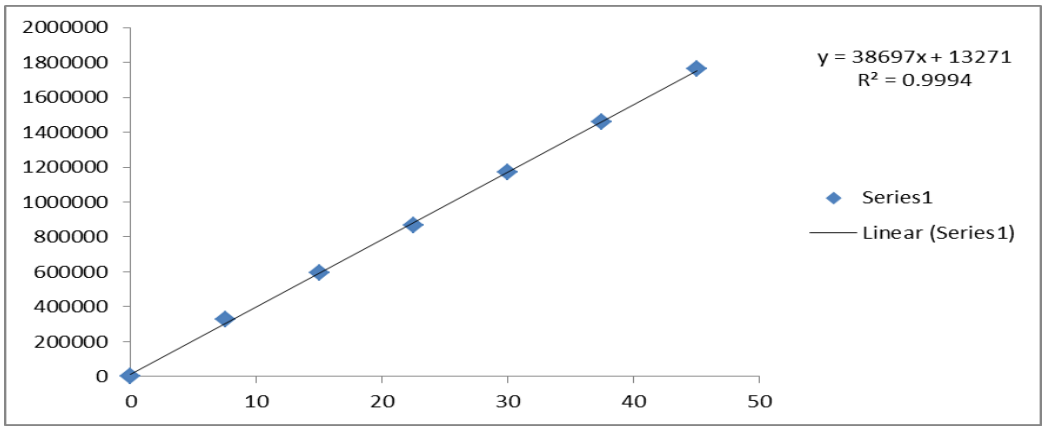

Fig. 8: Linearity curve of Lamivudine.

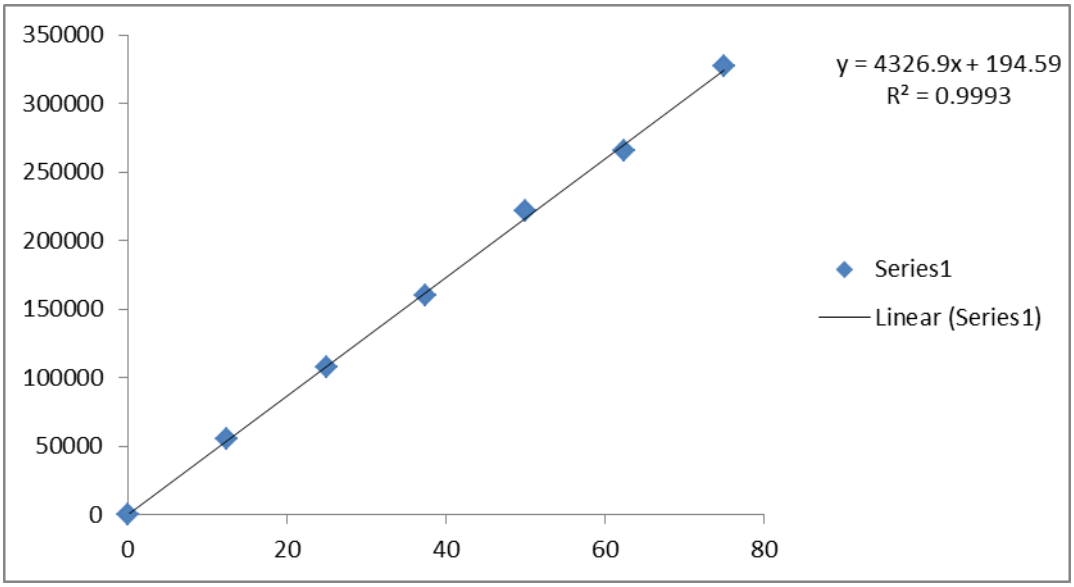

Fig. 9: Linearity curve of Dolutegravir.

Validation

The above optimized chromatographic method has been validated for the assay of ABC, LMD & DLT using the following parameters [International Conference on Harmonization (ICH) 1995]. Linearity was studied to find out the relationship of concentration with Peak area. Six different concentrations of Abacavir, Lamivudine and Dolutegravir (ABC, LMD & DLT) drug mixtures respectively. Each concentration of solution was injected into the HPLC and chromatogram was recorded. The calibration graph was constructed by plotting the peak versus the final concentration of the each drug (µg/ml) and the corresponding regression equation derived. Precision was studied to find out variations in the test methods of mixtures of Abacavir (30mg)+ Lamivudine (15mg)+ Dolutegravir (25mg) respectively. The precision of each method was ascertained separately from the peak area by actual determination of five replicates of a fixed amount of Abacavir (30mg)+ Lamivudine (15mg)+ Dolutegravir (25mg) respectively. The %RSD (percentage relative standard deviation) was calculated for precision and ruggedness. The accuracy of the method was shown by analyzing the model mixtures containing 80,100 and 120% of Abacavir, Lamivudine and Dolutegravir. After the measurement, the Amount found and individual recoveries were calculated. Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated based on the linearity data using the formulae LOD = 3.3×standard deviation /slope; LOQ = 10×standard deviation /slope. Robustness was performed by following the same method with different flow rate.

RESULTS AND DISCUSSION

The regression equation for ABC was found to be y = 37926x + 8085.1 (slope, intercept and correlation coefficient were found to be 37926, 8085.1 and 0.999 respectively) and linear over beer's range of 15-90 µg/ml. The regression equation for LMD was found to be y=38697 x+13271 (slope, intercept and correlation coefficient were found to be 38697, 13271 and 0.999 respectively) and linear over beer's range of 7.5-45 µg/ml. The regression equation for DLT was found to be y = 4326.9x + 194.59 (slope, intercept and correlation coefficient were found to be 4326.9, 194.59 and 0.999 respectively) and linear over beer's range of 12.5-75µg/ml. Linearity graph of ABC, LMD & DLT were shown in Figure 5, 6 & 7 respectively. Linearity data was shown in table 1. The precision and ruggedness were determined using the % RSD of the peak area for six replicate preparations of the drug. %RSD of system precision for Abacavir, Lamivudine and Dolutegravir were and found to be 1.1, 1.0 and 1.0 respectively. %RSD of method precision for Abacavir, Lamivudine and Dolutegravir were and found to be 0.4, 0.5 and 0.3 respectively. % recovery was obtained as 99.44%, 99.97% and 100.26% for Abacavir, Lamivudine and Dolutegravir respectively. The calculated RSD values were less than 2. Precision and ruggedness data are presented in Table 2. In order to verify the accuracy of the described method, recovery studies were carried out by analyzing model mixtures contained 50%, 100% and 150% of standard solution of drug ABC, LMD & DLT and along with 5 µg/mL of placebo solution within the linearity ranges. The mean percentage recoveries were found to be 99.44%, 99.97% and 100.26% w/w for 50%, 100% and 150% respectively. The results of accuracy were shown that the developed method have a good percentage recovery at different concentrations of drugs. LOD for ABC, LMD & DLT was found to be 0.04µg/ml, 0.11µg/ml and 0.08 µg/ml respectively. LOQ for ABC, LMD & DLT was found to be 0.13µg/ml, 0.33 µg/ml and 0.24 µg/ml respectively. Summary of all the validation parameter shown in table 6.

Degradation

Degradation studies were performed with the formulation and the degraded samples were injected.

Assay of the injected samples was calculated and all the samples passed the limits of degradation.

CONCLUSION

A simple, accurate, precise method was developed for the simultaneous estimation of the Abacavir, Lamivudine and Dolutegravir in Tablet dosage form was developed and the proposed method as suitable for routine analysis of ABC, LMD & DLT.

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