An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

HPLC METHOD DEVELOPMENT AND VALIDATION OF ABAKAVIR, LAMIVUDINE ANDDOLUTEGRAVIR IN HUMAN PLASMA

Padmavathi Sakinala*, PujithaG, Sharmila J, Sireesha M, Nikitha K

Department of Pharmaceutical Analysis, Pharmaceutical chemistry and phytochemistry, Nirmala college of pharmacy, Atmakur, Mangalagiri, Guntur, Andhrapradesh, A.P, India

*Corresponding author E-mail: padmavathi.sakinala@gmail.com

ARTICLE INFO

Key words:
Abacavir, Lamivudine and Dolutegravir; Emtricitabine; RP-HPLC; Method development: ICH Guidelines; validation.

Access this article online Website: https://www.jgtps.com/ Quick Response Code:

ABSTRACT

A simple, precised, accurate method was developed for the estimation of Abacavir, Lamivudine and Dolutegravir in human plasma using the Emtricitabine as internal standard by RP-HPLC (Reverse phase-High performance Liquid Chromatographic) technique. Chromatographic conditions used are stationary phase Azilent (250 x 4.6 mm, 5 □), Mobile phase 0.01N Potassium di-hydrogen phosphate (pH: 3.5) : Acetonitrile in the ratio of 70:30(v/v) and flow rate was maintained at 1.0ml/min, detection wave length was 250nm, column temperature was set to 30^{O}C and diluent was mobile phase Conditions were finalized as optimized method. Retention time of Lamivudine, Abacavir and Dolutegravir were found to be 3.225min, 3.594min and 5.229min. %CV of the Abacavir, Lamivudine and Dolutegravir was found to be 0.33%, 0.83% and0.49%.%Recovery was obtained as 98.23%, 99.96% and 97.59%. The linearity concentration is in the range of 220-8800ng/mL of Abacavir , 120-4800ng/mL of Lamivudine and 130- 5200ng/mL of Dolutegravir (r2 = 0.999) .The lower limits of quantification were 220ng/mLofAbacavir,120ng/mLofLamivudineand130ng/mLofDolutegravirwhich reach the level of both drugs possibly found in human plasma. Further, the reported method was validated as per the ICH guidelines and found to be well within the acceptable range.

INTRODUCTION

Bioanalytical techniques, employed for the quantitative determination of drugs and their metabolites in biological fluids and creates a specific procedure to enable a coalesce of interest to be identified and at the same time to be quantified in a matrix[1]. A coalesce is measured by several procedures[1]. The choice of analytical procedures involve many considerations, such as: concentration levels, chemical properties of the analyte, specimen matrix, cost of the analysis, Experimental speed, quantitative or qualitative measurement, required precision and necessary equipment[2]. Bioanalytical method validation comprises all criteria determining data quality, such as selectivity, accuracy, precision, recovery, sensitivity, and stability.[3] In this methods determine the drugs in biological fluid are becoming increasingly important for the study of bioavailability, bioequivalence (BE) Pharmacokinetics (PK) studies, quantitative evaluation of drugs, concentration and their metabolites, new drug development, research in basicbiomedicaland pharmaceutical sciences and therapeutic drug monitoring etc. High pressure liquid chromatography (HPLC) most widely applied analytical techniques because of its highly selective, and high reliability.

especially in pharmaceutical, environmental, food department, forensic and clinical department. A bioanalytical method is never fully validated, additional analytes may change methodology and revalidation requirements at any time during the drug development process. Bioanalysis covers the quantitative measurement of Xenobiotics of drugs such as their metabolites, and biological molecules in unnatural locations and Biotics like macromolecules proteins, DNA, large molecule drugs, metabolites in biological systems. Bioanalytical method validation includes all of the procedures that demonstrate that a particular method developed and used for quantitative measurement of analytes in a given biological matrix is reliable and reproducible. A simple, precised, accurate method was developed for the estimation of Abacavir, Lamivudine and Dolutegravir in human plasma using the Emtricitabine as internal standard by RP-HPLC (Reversephase-High performance Liquid Chromatographic) technique.

DRUG PROFILE

ABACAVIR: Abacavir (ABC) is a powerful nucleoside analog 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. In vivo, abacavir sulfate dissociates to its free base, abacavir.[1]

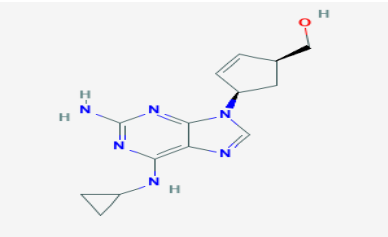


Fig 1: Structure of abacavir

LAMIVUDINE:A reverse transcriptase inhibitor and zalcitabine analog in which a sulfur atom replaces the 3' carbon of the pentose ring. It is used to treat Human Immunodeficiency Virus Type 1 (HIV-1) and hepatitis B (HBV).[2]

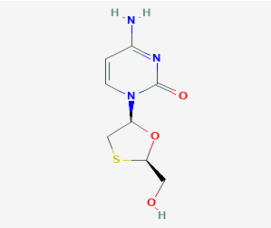


Fig 2: Structure of Lamivudine

DOLUTEGRAVIR: Dolutegravir is a HIV-1 intergrase inhibitor that blocks the strand transfer step of the integration of the viral genome into the host cell (INSTI).The effect of this drug has no homology in human host cells which gives it an excellent tolerability and minimal side effects

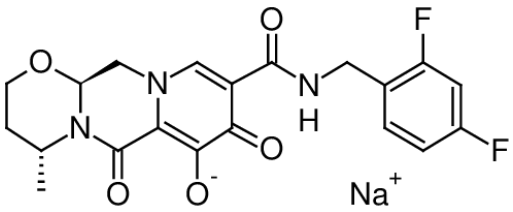


Fig 3: Structure of Dolutegravir

MATERIALS AND METHODS

Materials

API Abacavir, Doultegravir and Lamivudine was obtained as a gift sample from Spectrum pharma research solutions. Human plasma was obtained fromdecan pathological lab, Hyderabad. All the chemicals are obtained from Sigma Aldhric, Ranikem, Avantor performance materials , India. The HPLC used was Ajlance water 2695 system. Other instrument used are BVKenterprises.

METHOD DEVELOPMENT:

Diluent: Based up on the solubility of the drugs, diluent was selected, 0.01NPotassium dihydrogen phosphate and Acetonitrile taken in the ratio of 50:50.

Preparation of Abacavir Stock solution (1100 µg/ml): Take 110 mg of Abacavir in 100 ml volumetric flask and make the volume with diluent to produce 1100µg/ml.

Preparation of Abacavir Spiking Solutions:From the above Abacavir stock solution 0.05ml, 0.1ml, 0.15ml, 0.6ml, 1.0ml,

1.2ml, 1.6ml and 2.0 ml was pipette and transferred to 8 individual 25 ml volumetric flask and make up the volume up to the mark with diluent to produce 2.2 µg/ml, 4.4 µg/ml, 6.6µg/ml, 17.6 µg/ml, 44.0 µg/ml, 52.8 µg/ml, 70.4 µg/ml and 88.0 µg/ml.Calibration standards and quality control (QC) samples were prepared by spiking blank plasma with working stock dilutions of analytes to produce 220 ng/ml, 440 ng/ml, 660 ng/ml, 1760 ng/ml, 4400 ng/ml, 5280 ng/ml, 7040 ng/ml and 8800 ng/ml.

Preparation of Lamivudine Stock solution (600 µg/ml): Take 60 mg of Lamivudine in 100 ml volumetric flask and make the volume with diluent to produce 600 µg/ml.

Preparation of Lamivudine Spiking Solutions: From the above Lamivudine stock solution 0.05ml, 0.1ml, 0.15ml, 0.6ml, 1.0ml, 1.2ml, 1.6ml and 2.0 ml was pipette and transferred to 8 individual 25 ml volumetric flask and make up the volume up to the mark with diluent to produce 1.2 µg/ml, 2.4 µg/ml, 3.6µg/ml, 9.6 µg/ml, 24.0 µg/ml, 28.8 µg/ml, 38.4 µg/ml and 48.0 µg/ml.Calibration standards and quality control (QC) samples were prepared by spiking blank plasma with working stock dilutions of analytes to produce 120 ng/ml, 240 ng/ml, 360 ng/ml, 960 ng/ml, 2400 ng/ml, 2800 ng/ml, 3840 ng/ml and 4800 ng/ml.

Doultegravir

Preparation of Doultegravir Stock solution (650 µg/ml): Take 65 mg ofDoultegravir in 100 ml volumetric flask and make the volume with diluent to produce 650µg/ml.

Preparation of DoultegravirSpiking Solutions:From the above doultegravir stock solution 0.05ml, 0.1ml, 0.15ml, 0.6ml, 1.0ml, 1.2ml, 1.6ml and 2.0 ml was pipette and transferred to 8 individual 25 ml volumetric flask and make up the volume up to the mark with diluent to produce 1.3 µg/ml, 2.6 µg/ml, 3.9µg/ml, 10.4 µg/ml, 26.0 µg/ml, 31.2 µg/ml, 41.6 µg/ml and 52.0 µg/ml.Calibration standards and quality control (QC) samples were prepared by spiking blank plasma with working stock dilutions of analytes to produce 130 ng/ml, 260 ng/ml, 390 ng/ml, 1040 ng/ml, 2600 ng/ml, 3120 ng/ml, 4160 ng/ml and 5200 ng/ml.

Preparation of internal standard Solution (Emtricitabine):

Stock-1: Take 50 mg of Emtricitabine in 100 ml volumetric flask and make up the volume with diluent to produce 500µg/ml.

Stock-2: From the above solution, take 1ml of solution into 10 ml volumetric flask and make up the volume with diluent to produce 50µg/ml solutions.

Final concentration: From the above solution, take 0.5ml of solution and spiking blank plasma with working stock dilutions of analyte to produce 10µg/ml ISD concentration.

Extraction procedure: Take 750µl of plasma and 500µl of internal standard, 250µl of Abacavir from the spiking solutions of both into a centrifuging tube and add 1 ml of Acetonitrile go for cyclomixer for 15 sec. Then vertex for 2 min and finally centrifuge for 5 min at 3200 rpm speed. After the centrifugation collect the sample and filter it directly inject 10 µL into HPLC.

750µl of plasma +500µl of internal standard, 250µl of Abacavir.

RESULTS AND DISCUSSION

Method development:

The chromatographic column used was Azilent (250 x 4.6 mm, 5 m), Mobile phase 0.01N Potassium di-hydrogen phosphate (pH: 3.5) : Acetonitrile in the ratio of 70:30(v/v) and flow rate was maintained at 1.0ml/min. The column temperature was maintained at 30°C and the detection was monitored at a wavelength of 250 nm. The total chromatographic runtime is 12.0 min with Retention time of Lamivudine, Abacavir and

Dolutegravir were found to be 3.225min, 3.594min and 5.229min. (Table1)

Method Validation:

The optimized chromatographic method was completely validated according to the procedures described in USFDA guidelines for the validation of analytical methods and Stability Testing of New Drug, respectively.

System Suitability:

All the system suitability parameters were within the range and satisfactory as per ICH guidelines.

The % CV for system suitability test was in the range of 0.38–0.46 for Retention time (RT) of abacavir, 0.38–0.10 for Retention time (RT) of Lamivudine, 0.38–1.54 for Retention time (RT) of Dolutegravir and 0.33% for the area ratio (analyte area/IS area) of abacavir, 0.83% for the area ratio (analyte area/IS area) of Lamivudine and 0.49% for the area ratio (analyte area/IS area) of Dolutegravir.

Selectivity: To establish the selectivity of the method, possible interference at the retention time of Abacavir, Lamivudine and Dolutegravir and Internal standard due to endogenous plasma components were checked during the validation. Selectivity was performed by testing six batches of K2EDTA blank plasma and the mass detection of extracted blank plasma gave good selectivity of both drug and internal standard. Representative chromatograms of standard blank and blank with internal standard sample using pooled plasma. This result was shown in Fig no: 1.4.1.5, 1.6.

Sensitivity: Six LLOQ samples were prepared independent of calibration curve standards. Data was represented in table 24,25,26. The % CV of area was found to be 1.30 for Abacavir and

1.88 for Doultegravir) and 4.18 for Lamivudine within the acceptable limit.

Linearity and Calibration Curves: Calibration was found to be linear over the concentration range of 220 to 8800 ng /ml for abacavir, 120 to 480ng/ml Lamivudine and 130 to 5200 ng/ml of Dolutegravir. The coefficient correlation (r2) value was found consistently greater than 0.999 in all the cases. This indicating linearity of results and an excellent correlation between peak area ratios for each concentration of analyte

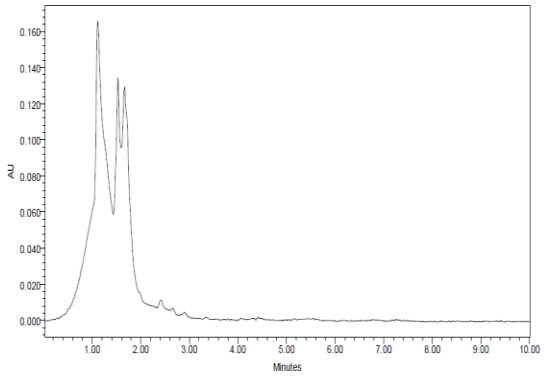
A representative calibration curve is shown in Figure 25, which is obtained during the third precision and accuracy batch. Back calculated concentrations obtained for 3 calibration curves are summarized in the fig 1.7, 1.8, 1.9. table-2,3,4.

Precision and Accuracy Studies:

The intraday and inter day accuracy and precision was assessed by analysing six replicates at five different QC levels like LLOQ, LQC, MQC and HQC. Accuracy and precision method performance was evaluated by determined by six replicate analyses for Abacavir at four concentration levels, i.e., 660ng/ml (LQC), 4400ng/ml (MQC) and 7040ng/ml (HQC), Lamivudine at 360ng/ml (LQC), 2400ng/ml (MQC) and Dolutegravir at 390ng/ml (LQC), 2600ng/ml (MQC) and 4160ng/ml (HQC), The intra-day and inter day accuracy of plasma samples were assessed and excellent mean % accuracy was obtained with range varied from 100.00%-100.75%, 100.00%-100.85% and 100.00%-100.33% for intraday and 99.79%-100.04, 100.05%-10.59 and 100.00%-100.70 for inter day respectively. The precision (%CV) of the analytes and plasma samples were calculated and found to be 0.1%-2.40%, 0.18%-3.60% and 0.12%-2.94% for intraday and 0.11%-2.74%, 0.21%-3.20% and 0.12%-3.67% for inter day respectively.

Ruggedness:

Ruggedness was performed by different analyst using or different column. The run consisted of a calibration curve and a total of 6 spiked samples; six replicate each of the LLOQ, LQC, MQC, HQC samples. Thewithin-run% coefficients of variation ranged from to 1.30 for Abacavir,0.14 to 1.88 for Doultegravir and 0.14 to 4.18 for Lamivudine.



FigNo 1.4 : Extracted Standard Blank Sample

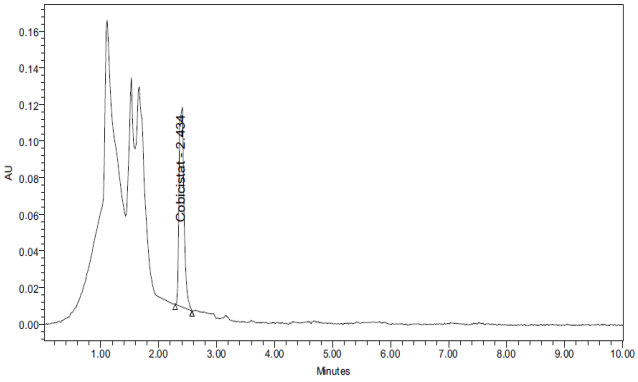
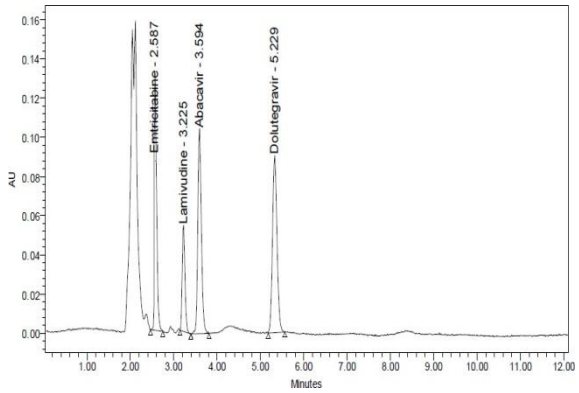


Fig No 1.5 Chromatogram of Pure Drug



(Cobicistat)

Fig 1.6: Optimized Chromatogram

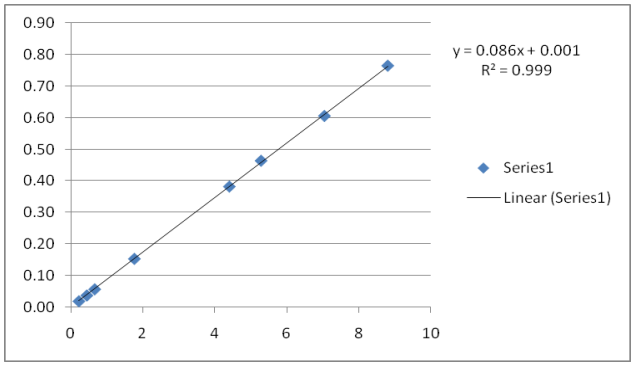


Fig.1.7. Calibration Curve of Abacavir

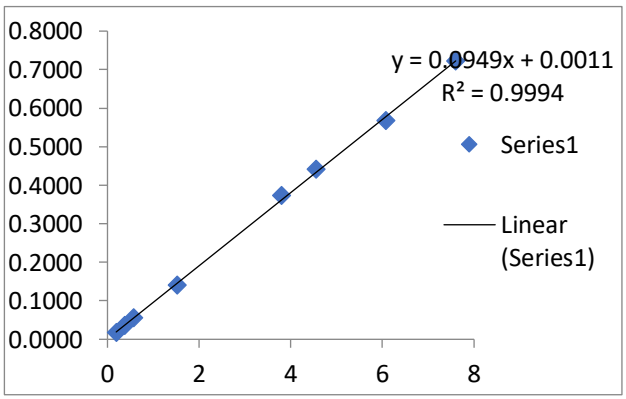


Fig.1.8- Calibration Curve of Lamivudine

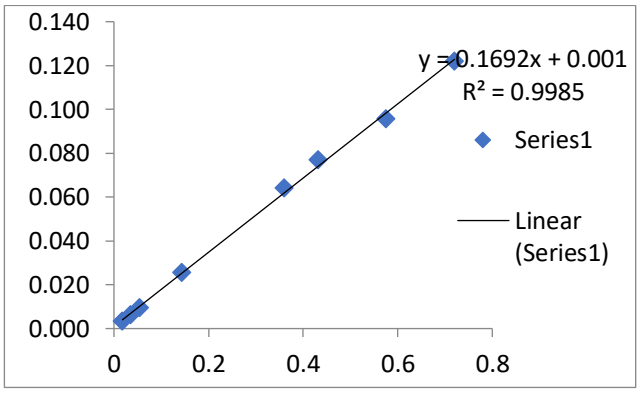


Fig.1.9- Curve of Doultegravir

Name	Parameters
Column	Azilent (250 x 4.6 mm, 5 m),
Mobile phase composition	N Potassium di-hydrogen phosphate (pH: 3.5) : Acetonitrile in the ratio of 70:30(v/v)

Table 1: Optimized chromatographic conditions

Flow rate	1 ml/min
Injection volume	10 µl
Run time	12.0 min
Detection wavelength	250nm
Column temperature	30 ºc
Diluent	water : Acetonitrile 50:50
Flow rate	1 ml/min
Injection volume	10 µl
Run time	12.0 min
Detection wavelength	250nm
Column temperature	30 ºc
Diluent	water : Acetonitrile 50:50

Table 2 - System suitability of Abacavir

Sample Name	File Name	Analyte Area	Analyte RT (min)	ISTD Area	ISTD RT (min)	Area Ratio
AQ MQC		321798	3.537	847903	2.565	0.3795
AQ MQC		320518	3.538	845621	2.565	0.3790
AQ MQC		325607	3.541	852039	2.571	0.3822
AQ MQC		324125	3.544	849012	2.573	0.3818
AQ MQC		325502	3.556	853603	2.584	0.3813
AQ MQC		326204	3.562	856122	2.588	0.3810
MEAN			4.695		2.574	0.38080
SD			0.0218		0.0097	0.001252
%CV			0.46		0.38	0.33

Table 3 - System Suitability of Dolutegravir

Sample Name	File Name	Analyte Area	Analyte RT (min)	ISTD Area	ISTDRT (min)	Area Ratio
AQ MQC		276355	4.852	847903	2.565	0.3259
AQ MQC		277496	4.856	845621	2.565	0.3282
AQ MQC		278187	4.964	852039	2.571	0.3265
AQ MQC		275747	4.972	849012	2.573	0.3248
AQ MQC		276411	5.019	853603	2.584	0.3238
AQ MQC		277742	5.023	856122	2.588	0.3244
MEAN			4.948		2.574	0.32560
SD			0.0764		0.0097	0.001593
%CV			1.54		0.38	0.49

Table 4- System Suitability of lamivudine

Sample Name	File Name	Analyte Area	Analyte RT (min)	ISTD Area	ISTDRT (min)	Area Ratio
AQ MQC		132148	3.133	847903	2.565	0.1559
AQ MQC		132250	3.133	845621	2.565	0.1564
AQ MQC		131636	3.134	852039	2.571	0.1545
AQ MQC		130215	3.136	849012	2.573	0.1534
AQ MQC		132155	3.138	853603	2.584	0.1548
AQ MQC		131170	3.141	856122	2.588	0.1532
MEAN			3.136		2.574	0.15469
SD			0.0032		0.0097	0.001283
%CV			0.10		0.38	0.83

Acquisition Batch ID						
Replicate No.	**HQC**		**MQC1**		**LQC**	
	Un extracted Response	Extracted Response	Un extracted Response	Extracted Response	Un extracted Response	Extracted Response
1	214568	205871	135019	130181	20269	19482
2	219240	200267	134871	131972	19932	19543
3	215073	204643	133891	133984	20075	20011
4	216151	203263	134590	134452	20794	19713
5	213793	201738	135314	134494	19778	20096
6	215572	199993	133981	133643	20014	19688
N	6	6	6	6	6	6
Mean	215733	202629	134611	133121	20144	19756
SD	1900.32	2378.66	573.41	1711.01	357.40	248.01
% CV	0.88	1.17	0.43	1.29	1.77	1.26
% Mean Recovery	106.47		98.89		98.07	
Overall % Mean Recovery	96.964					
Overall SD	2.6628					
Overall % CV	2.75					

Table 5 - Recovery of Lamivudine

Acquisition Batch ID						
Replicate No.	HQC		MQC1		LQC	
	Un extracted Response	Extracted Response	Un extracted Response	Extracted Response	Un extracted Response	Extracted Response
1	214568	205871	135019	130181	20269	19482
2	219240	200267	134871	131972	19932	19543
3	215073	204643	133891	133984	20075	20011
4	216151	203263	134590	134452	20794	19713
5	213793	201738	135314	134494	19778	20096
6	215572	199993	133981	133643	20014	19688
N	6	6	6	6	6	6
Mean	215733	202629	134611	133121	20144	19756
SD	1900.32	2378.66	573.41	1711.01	357.40	248.01
% CV	0.88	1.17	0.43	1.29	1.77	1.26
% Mean Recovery	106.47		98.89		98.07	
Overall % Mean Recovery	96.964					
Overall SD	2.6628					
Overall % CV	2.75					

Table no 6 Recovery of Doltugravir

Acquisition Batch ID						
Replicate No.	HQC		MQC1		LQC	
	Un extracted Response	Extracted Response	Un extracted Response	Extracted Response	Un extracted Response	Extracted Response
1	564957	549744	285043	275216	42501	41323
2	567613	544559	288329	272257	42829	41085
3	560607	548609	282064	280005	43071	42377
4	559011	549942	279808	276302	42236	42059
5	570017	548880	286135	275152	42006	41956
6	571696	549299	280784	277821	42780	42366
N	6	6	6	6	6	6
Mean	565650	548506	283694	276126	42571	41861
SD	5088.55	1997.64	3331.29	2634.26	399.15	540.58
% CV	0.90	0.36	1.17	0.95	0.94	1.29
% Mean Recovery	96.97		97.33		98.33	
Overall % Mean Recovery	97.545					
Overall SD	0.7066					
Overall % CV	0.72					

Table no7: Recovery of Abacavir

Acquisition Batch ID						
Replicate No.	HQC		MQC1		LQC	
	Un extracted Response	Extracted Response	Un extracted Response	Extracted Response	Un extracted Response	Extracted Response
1	676966	654529	330085	322892	50663	50812
2	670244	649578	329903	326956	51869	50197
3	677903	653478	329989	321088	50103	49595
4	672983	655034	329329	324258	51009	49961
5	672214	656555	330475	329398	50661	50694
6	672148	654625	331451	325950	50193	50784
N	6	6	6	6	6	6
Mean	673743	653967	330205	325090	50750	50341
SD	3013.16	2370.13	713.45	2977.24	642.43	502.86
% CV	0.45	0.36	0.22	0.92	1.27	1.00
%Mean Recovery	97.06		98.45		99.19	
Overall % Mean Recovery	98.236					
Overall SD	1.0806					
Overall % CV	1.10					

Table no 8 - Matrix samples stability at -28±5 ^{0}C for 37 days (Abacavir)

Acquisition BatchID			Date			
Replicate No.	HQC			LQC		
	Nominal Concentration (ng/mL)					
	7040.000	7040.000		660.000		660.000
	Nominal Concentration Range (ng/mL)					
	(5,984.000-8,096.000)	(5,984.000-8,096.000)		(561.000-759.000)		(561.000-759.000)
	Calculated C oncentration (ng/mL)					
	Comparison Samples	Stability Samples		Comparison Samples		Stability Samples
1	7043.540	7034.120		652.755		663.779
2	7041.320	7032.210		650.790		661.718
3	7037.960	7038.030		657.715		654.789
4	7046.840	7046.940		660.810		662.859
5	7045.590	7047.580		662.820		659.689
6	7042.800	7043.890		666.691		667.668
N	6	6		6		6
Mean	7043.0083	7040.4617		658.5968		661.7503
SD	3.16256	6.61230		6.06795		4.31448
% CV	0.04	0.09		0.92		0.65
%Mean Accuracy	100.04	100.01		99.79		100.27
%Mean Stability	99.96			100.48		

Table no 9 - Matrix samples stability at -28±5 ^{0}C for 37days(Doultegravir)

Acquisition Batch ID			**Date**	
Replicate No.	HQC		LQC	
	Nominal Concentration (ng/mL)			
	5200.000	5200.000	390.000	390.000
	Nominal Concentration Range (ng/mL)			
	(4,420.000-5,980.000)	(4,420.000-5,980.000)	(331.500-448.500)	(331.500-448.500)
	Calculated Concentration (ng/mL)			
	Comparison Samples	Stability Samples	Comparison Samples	Stability Samples
1	5193.540	5194.120	392.755	384.779
2	5191.320	5192.210	390.790	383.718
3	5197.960	5198.030	384.715	387.789
4	5203.840	5206.940	387.810	392.859
5	5205.590	5207.580	394.820	390.689
6	5206.800	5203.890	396.691	394.668
N	6	6	6	6
Mean	5199.8417	5200.4617	391.2635	389.0837
SD	6.53175	6.61230	4.45791	4.40481
% CV	0.13	0.13	1.14	1.13
%Mean Accuracy	100.00	100.01	100.32	99.77
%Mean Stability	100.01		99.44	

Table no 10 - Matrix samples stability at -80±5 ^{0}C for 37days(Lamivudine)

Acquisition Batch ID			Date	
Replicate No.	HQC		LQC	
	Nominal Concentration (ng/mL)			
	7040.000	7040.000	660.000	660.000
	Nominal Concentration Range (ng/mL)			
	(5,984.000-8,096.000)	(5,984.000-8,096.000)	(561.000-759.000)	(561.000-759.000)
	Calculated Concentration (ng/mL)			
	Comparison Samples	Stability Samples	Comparison Samples	Stability Samples
1	7042.697	7035.798	652.784	650.795
2	7040.200	7032.345	654.830	658.779
3	7037.894	7038.895	666.750	662.748
4	7035.325	7043.285	663.821	666.668
5	7033.592	7040.992	660.680	664.810
6	7045.062	7041.725	661.715	668.696
N	6	6	6	6
Mean	7039.1283	7038.8400	660.0967	662.0827
SD	4.37566	4.10254	5.33497	6.49566
% CV	0.06	0.06	0.81	0.98
%Mean Accuracy	99.99	99.98	100.01	100.32
%Mean Stability	100.00		100.30	

Table no 11 - Matrix samples stability at -80±5 ^{0}C for 37days (Abacavir)

Acquisition Batch ID			Date	
Replicate No.	HQC		LQC	
	Nominal Concentration (ng/mL)			
	3840.000	3840.000	360.000	360.000
	Nominal Concentration Range (ng/mL)			
	(3,264.000-4,416.000)	(3,264.000-4,416.000)	(306.000-414.000)	(306.000-414.000)
	Calculated Concentration (ng/mL)			
	Comparison Samples	Stability Samples	Comparison Samples	Stability Samples
1	3843.540	3834.120	362.755	363.779
2	3841.320	3832.210	360.790	361.718
3	3837.960	3838.030	364.715	364.789
4	3833.840	3846.940	357.810	352.859
5	3845.590	3847.580	350.820	359.689
6	3846.800	3853.890	346.691	357.668
N	6	6	6	6
Mean	3841.5083	3842.1283	357.2635	360.0837
SD	4.90345	8.60828	7.09525	4.39667
% CV	0.13	0.22	1.99	1.22
%Mean Accuracy	100.04	100.06	99.24	100.02
%Mean Stability	100.02		100.79	

Table no.12: Matrix samples stabilityat-80±5 for Doultegravir

Acquisition Batch ID			Date	
Replicate No.	HQC		LQC	
	Nominal Concentration (ng/mL)			
	3840.000	3840.000	360.000	360.000
	Nominal Concentration Range (ng/mL)			
	(3,264.000-4,416.000)	(3,264.000-4,416.000)	(306.000-414.000)	(306.000-414.000)
	Calculated Concentration (ng/mL)			
	Comparison Samples	Stability Samples	Comparison Samples	Stability Samples
1	3842.697	3845.798	368.784	360.795
2	3840.200	3842.345	364.830	358.779
3	3837.894	3838.895	366.750	362.748
4	3847.325	3843.285	363.821	356.668
5	3843.592	3840.992	357.680	364.810
6	3835.062	3836.725	351.715	358.696
N	6	6	6	6
Mean	3841.1283	3841.3400	362.2633	360.4160
SD	4.36010	3.22689	6.38651	2.98579
% CV	0.11	0.08	1.76	0.83
%Mean Accuracy	100.03	100.03	100.63	100.12
% Mean Stability	100.01		99.49	

Table no.13: Matrix samples stabilityat-80±5 for Lamivudine

The percentage of nominal values ranged from 99.90 to 100.02 for Abacavir and 99.06 to 100.49 forDoultegravir.and 100.00 to 101.90 for Lamivudine .

Recovery: Recovery was determined by measuring the peak areas obtained from prepared plasma samples with those extracted blank plasma spiked with standards containing the same area with known amount of Abacavir, Lamivudine and Dolutegravir. The overall % mean recovery for Abacavir, Lamivudine and Dolutegravir was found to be 98.23%,99.96% and 97.54%.The recoveries obtained for Abacavir, Lamivudine and Dolutegravir at 3 QC concentration levels are summarized in the Table no:5,6,7.The overall % mean recovery for Emtricitabine was found to be 98.02%. The recovery results of Emtricitabine are Matrix samples stability at -28±5 □C for 37 days & -80±5 ^{0}C. Long term stock solution stability for the Abacavir was determined at a concentration of LQC-HQC level after a storage period of 37 days at -28°C& -80°C in refrigerator. The % mean stability of the Abacavir was found to be 100.04%, 100.01% & 99.79%, 100.01 at 28 ± 5°C and 99.99%, 99.98% &100.01%, 100.32 at 80 ± 5°C respectively. Long term stock solution stability for the Lamivudine was determined at a concentration of LQC-HQC level after a storage period of 37 days at -28°C& -80°C in refrigerator. The % mean stability of the Lamivudine was found to be 100.04%, 100.06% &99.24%, 100.02% at 28± 5°C and 100.03%, 100.03% &100.63%, 100.12 at 80 ± 5°C respectively. Long term stock solution stability for the Dolutegravir was determined at a concentration of LQC-HQC level after a storage period of 37 days at -28°C& -80°C in refrigerator. The % mean stability of the Dolutegravir was found to be 100.00%, 100.01% &100.32%, 99.77% at 28± 5°C and 99.99%, 99.98% &99.60%, 100.11 at 80 ± 5°C respectively table no 8,9,10,11,12,13.The long term stability of Abacavir, Lamivudine and Dolutegravir is presented in the Table.

CONCLUSION:

A simple, accurate, precise method was developed for the estimation of the Abacavir,

Lamivudine and Dolutegravir in human plasma using the Emtricitabine as internal standard. Retention time of Lamivudine, Abacavir and Dolutegravir were found to be 3.225min, 3.594min and 5.229min. %CV of the Abacavir, Lamivudine and Dolutegravir was found to be 0.33%, 0.83% and 0.49%. %Recovery was obtained as 98.23%, 99.96% and 97.59% The linearity concentration is in the range of 220-8800ng/mL of Abacavir, 120-4800ng/mL of Lamivudine and 130-5200ng/mL of Dolutegravir (r^2 = 0.999) . The lower limits of quantification were 220ng/mL of Abacavir , 120ng/mL of Lamivudine and 130ng/mL of Dolutegravir which reach the level of both drugs possibly found in human plasma. Further, the reported method was validated as per the ICH guidelines and found to be well within the acceptable range. The proposed method is simple, rapid, accurate, precise, and appropriate for pharmacokinetic and therapeutic drug monitoring in the clinical laboratories.

REFERENCES:

1. De Clercq E: New anti-HIV agents and targets. Med Res Rev. 2002 Nov;22(6):531-65.
2. Minuesa: Transport of lamivudine [(-)-beta-L-2',3'-dideoxy-3'-thiacytidine] and high-affinity interaction of nucleoside reverse transcriptase inhibitors with human organic cation transporters 1, 2, and 3. J Pharmacol Exp Ther. 2009 Apr;329(1):252-61. doi: 10.1124/jpet.108.146225. Epub 2009 Jan13
3. Dow DE, Bartlett JA: Dolutegravir, the Second-Generation of Integrase Strand Transfer Inhibitors (INSTIs) for the Treatment of HIV. Infect Dis Ther. 2014 Dec;3(2):83-102. doi: 10.1007/s40121-014-0029-7. Epub 2014 Jun24
4. Ramesh Adepu,et,al. A High Sensitive Method for the Simultaneous Determination of Abacavir and Lamivudine in Human plasma by using Liquid chromatographyelectro spray ionization tandem mass spectrometry and application to a pharmacokinetic study, JCPS ,2017, 10(4).
5. Chantelle Bennetto-Hood,et,al. A Sensitive HPLC-MS/MS Method for the Determination of Dolutegravir in Human Plasma, J Chromatogr B Analyt Technol Biomed Life Sci. 2014 Jan 15; 0:225–232.
6. Sindu,et,al. simultaneous stability-indicating method for the determination of abacavir, dolutegravir and lamivudine by RP-HPLC, Int J Pharm Sci Res 2016; 7(7):2905-16.
7. Somshankardubey,et,al. Simultaneous estimation of lamivudine, abacavir and dolutegravir by HPLC method, Int J App Pharm, 2018, 10(1),46-52.
8. Rajkumar Prava,et,al. RP-HPLC method development and validation for the simultaneous determination of Lamivudine, Abacavir and Dolutegravir in pharmaceutical dosage forms, World J Pharm Sci 2017; 5(5):168-181.
9. GorjaAshok,et,al. Development and Validation of Stability Indicating Method for the Simultaneous Estimation of m in Pharmaceutical Dosage forms by RP- HPLC, Saudi J. Med. Pharm. Sci., 2018 , 4(2):289-296.
10. M.Monica,et,al.Simultaneous RP-HPLC determination of Abacavir and Lamivudine and Doultegravir in bulk API dosage forms,IJPAR 2015,4(3).391- 398.
11. Bommakanti Valli Purnima,et al. Validated Reversed Phase HPLC Method for Assay and Degradation Studies of Lamivudine, Abacavir Sulphate And Dolutegravir In Combined Dosage Form, IJEAR,2016, 6, (2).
12. NagarajuPappula,et al. stability indicating HPLC method for simultaneous estimation of lamivudine, abacavir and dolutegravir from its tablet dosage form, IJPCBS 2015, 5(1),63-7