



**DEVELOPMENT AND VALIDATION OF SIMPLE, SELECTIVE AND
SENSITIVE LIQUID CHROMATOGRAPHY TANDEM MASS
SPECTROSCOPY METHOD FOR QUANTIFICATION OF
DOLUTEGRAVIR IN HUMAN PLASMA**

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ABSTRACT

A simple, rapid, sensitive and novel technique for quantifying of Dolutegravir in human plasma was developed and validated. A protein precipitation technique was used as sample preparation method and Tenofovir is employed as Internal standard (IS). Chromatographic condition was conducted using a Agilent, Zorbax, XDB C18 2.1 × 50 mm, 5 µm., 5 Micro meter, and a Mobile Phase (70: 30) of proportion Acetonitrile;1% Formic acid delivered at 1.20 ml/min and injection volume of 10 µL with run time of 3 minutes. ESI-MS with positive ion (PI) mode at atmospheric pressure was employed to carry out detection. The validated Linearity range is 2ng to 1000ng/ml with correlation value of 0.998 , excellent accuracy and precision was obtained. The retention time of Dolutegravir and Tenofovir 1.37 and 1.20 min.

KEY WORDS: Dolutegravir, Tenofovir ,Protein precipitation, Internal Standard.

INTRODUCTION

Dolutegravir is an Antiretroviral agent prescribed for treating HIV/AIDS, and also treat post exposure prophylaxis, it blocks the HIV Integrase which cause viral replication is prescribed along with other drugs for managing HIV infection in adults and in infants above 1 month of age weighing not less than 3 kg. DLG is prescribed in conjunction with rilpivirine as a replacement with their current HIV drugs, and given for a minimum of 6 months. DLG is a HIV integrase inhibitors¹. It works by boosting the quantity of immune cells that combat infections and decreases the amount of HIV in your blood and in your body. Although dolutegravir alone does not fight HIV, however, when combined with other drugs, it may

lower the risk of getting AIDS and HIV-linked diseases like severe infections or cancer².DLG attaches to the binding site of integrase, an enzyme responsible for initiation of the incorporation of viral genome into host chromosomes upon taking by oral route. As a result, the strand transferring phase, which is necessary for the viral replication process, is blocked and integrase is unable to attach to retroviral DNA. This stops the replication of HIV-1³.Dolutegravir is more effective and with few side effects⁴.

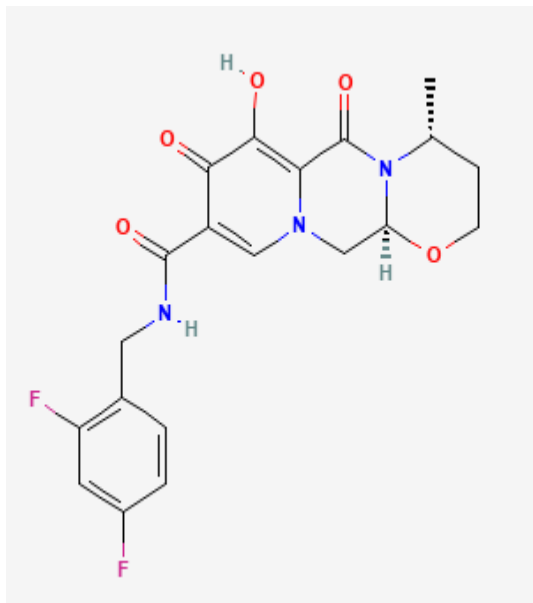


Figure 1: Chemical structure of Dolutegravir

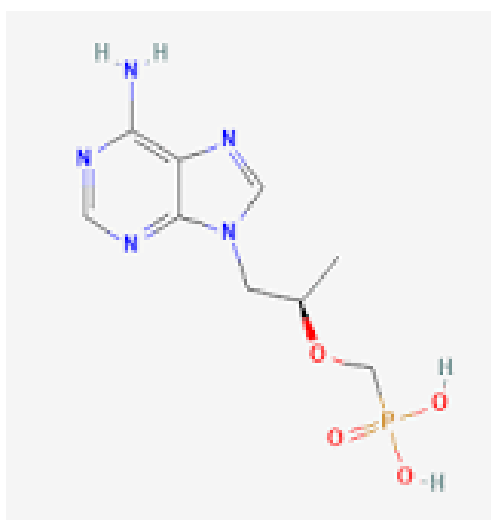


Figure 2: Chemical Structure of Tenofovir

The use of therapeutic drug monitoring (TDM) for Anti-Viral drugs can help patients with HIV achieve better clinical and safety outcomes⁵.In cases of drug-drug interactions,

pharmacokinetic alterations, for instance during gestation or in individuals with kidney and liver dysfunction, monitoring drug levels can also be helpful. Due to this, as well as the considerable quantity of research involving INSTIs and NNRTIs, it is necessary to have an exact, precise, and validated analytical approach⁷.

2.MATERIALS AND METHODS:

Drugs and Reagents:

Dolutegravir and Tenofovir (IS) are obtained from Fisher Chemicals, Mumbai, India. The acetonitrile (ACN) was acquired from Rankem Ltd., India. Merck Specialties Pvt.Ltd., Mumbai, India, provided HPLC grade water, formic acid and ammonium acetate.

Instrument:

A LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROSCOPY a Waters Acquity UPLC system integrated with a Water Quattro Premier XE mass spectroscope with ESI was employed for analyzation and Mass Lynx 4.1 SCN 805 software was employed for data acquisition and processing. Agilent, Zorbax, XDB C18 2.1 × 50 mm, 5 μm column was utilized as stationary phase.

Bio-analytical conditions:

By employing a mobile phase comprising a combination of 5 mM Ammonium Formate buffer (AFB) with 0.1% formic acid (FA): ACN in 70:30, v/v, delivered at 0.120 mL/min, the technique was adopted for quantifying Dolutegravir. Detector used was atmospheric pressure ESI-MS in positive ion mode (API 4000).

1.1 Bio Analytical Method

An outline of the HPLC and MS conditions are given below:

HPLC	:	Waters Acquity UPLC
MASS	:	Waters Quattro Premier XE
Ion source	:	Electrospray ionization (ESI)
Detection ions (m/z)		
Polarity	:	Positive ion mode
Dolutegravir	:	420.28* amu (parent), 277.05* amu (product)
Tenofovir	:	477.20* amu (parent), 270.00* amu (product)

Column	:	Agilent, Zorbax, XDB C18 2.1 × 50 mm, 5 μm.
Column oven temp.	:	30 °C
Autosampler temp.	:	10 °C
Mobile phase	:	Mobile Phase (70: 30) of proportion A: ACN& B: 0.1% FA in water
Flow rate	:	0.120 mL/min
Injection volume	:	10 μL
Retention time (RT)	:	Dolutegravir : 1.37 Tenofovir : 1.20
Run time	:	3.0 minutes
Diluent	:	Methanol: Water (50: 50)
Linearity Range	:	2ng-1000ng
This parameter may change by 0.5 units.		
MRM Conditions**		
Positive ion mode:		
Capillary (KV)	:	3.00
Extractor (V)	:	3
Source Temp.	:	120 °C
Desolvation Temp.	:	400 °C
Cone Gas Flow(L/Hr)	:	100
LM1 Resolution	:	15
HM1 Resolution	:	15
Multiplier (V)	:	550

1.2 Procedure for sample preparation

Step 1:

Plasma blanks and Quality Control (QC) samples were taken out of the freezer, brought to ambient temperature, and then refluxed. 20 μL of 50% MeOH in water were added, and the vial was assigned a 'blank' label. Transferred 100 μL of sample out

from allocated samples into the pre-labeled vials and refluxed. 20 μ L of ISTD (combined ISTD with around 2 μ g of Tenofovir) was added.

Step 2: 0.300 mL of ACN was added, refluxed, and centrifugated at a speed of 4,000 rpm while the temperature was above 20°C. 150 μ L of the supernate was then put into auto-injector vials; 10 μ L was loaded into an LC-MS/MS column.

Standard and working solutions for Dolutegravir

Dolutegravir stock solution was produced by combining 10 mg of Dolutegravir with 10 mL of mobile phase to acquire a 1000 μ g/mL solution. This mixture was stored in the fridge between 2 and 8 °C. To produce calibration curve standards and QC samples for subsequent usage, the above solutions were reconstituted to the proper dilutions by adding mobile phase before being spiked into plasma. The mobile phase was used to generate all other serial dilution.

Stock solution for Tenofovir

Tenofovir stock solution was obtained by combining 10 mg of Tenofovir with 10 mL of mobile phase for acquiring a 1000 μ g/ml solution. This mixture was stored in the fridge between 2 and 8 °C. Working IS solutions were obtained by appositely reconstituting the prepared stock solution freshly prior to its usage.

Calibration curve standards and QC samples

Dolutegravir calibration curve standards were obtained using a series of 8 concentrations, varying between 2-1000 ng/mL. The lower LOQ QC sample, LQC, MQC, and HQC had a concentration of 0.2, 2, 480, and 750 ng/mL, correspondingly. Before usage, these samples were maintained at -70 ± 10 °C. To test for stability, 12 series of LQC and HQC samples were kept at -20 ± 5 °C.

Plasma samples

Blood samples were drawn and placed into K2-EDTA-containing polypropylene tubes for obtaining plasma samples. The supernate from every tube was deposited in a separate tube after centrifuging them for 15 minutes at 4500 rpm. 1 ml of ACN was added to the collected supernate, which was then maintained for 10 min to allow for precipitation of plasma proteins. The supernate was preserved for future use.

Method for Spiked Human Plasma

Protein precipitation extraction(PPE) technique was employed for extracting Dolutegravir and Tenofovir (Internal Standard) from plasma samples. To achieve this, 100 μL of plasma sample and 20 μL of IS were divided into dilutions and refluxed in labelled polypropylene tubes. To it 20 μL of 0.1% FA and was added and agitated thoroughly. 5 mL of ammonium formate acetate was added, and the solution was agitated for 30 minutes at 500 rpm using a reciprocating shaker. At 5 $^{\circ}\text{C}$, samples were centrifugated for 10 minutes at 2000 rpm. The supernate organic layer (5.0 mL) was collected in pre-labelled test tubes and vaporized in a turbovapor at 40 $^{\circ}\text{C}$ until dry. 1000 μL of mobile phase was added for further dilution the solution and 10 μL of this solution was loaded into column with MS-MS detection.

Preparation of sample solution

Following bulk spiking, 100 μL dilutions each, for obtaining calibration curve and for QC of the spiked samples, were transferred to pre-labeled tubes. Each of the bulk spiked sample was preserved in a fridge at -70 ± 10 $^{\circ}\text{C}$, with the exception of 12 replicates of LQC as well as HQC; they were preserved at -20 ± 5 $^{\circ}\text{C}$. To confirm that the components were well mixed, the thawed samples were mixed thoroughly.

LC/MS/MS technique optimization

Numerous parameters including mobile phase ratios were tested for the LC-MS/MS technique's optimization. Good separation and no interference of the chromatographic peaks was observed by utilizing Agilent, Zorbax, XDB C18 (2.1 x 50 mm ID, 5 μm) plus a mobile phase comprising a combination of 5 mM AFB with 0.1% FA: ACN in 70:30, v/v, supplied at 0.120 mL/min with injecting volume of 10 μL as well as a run duration of 3 min, an acceptable LC-MS chromatography technique was adopted for quantifying Dolutegravir and findings indicated excellent peak symmetry. Detector used was atmospheric pressure ESI-MS in positive ion mode (API 4000). The precursor to product ion transitions is m/z 420.28>277.05 for Dolutegravir and m/z 477.20 to 270.00 for Tenofovir were utilized for analysis, as depicted in Figure 3 and 4. The RT of Dolutegravir and Tenofovir was determined to be 1.37 & 1.20 min.

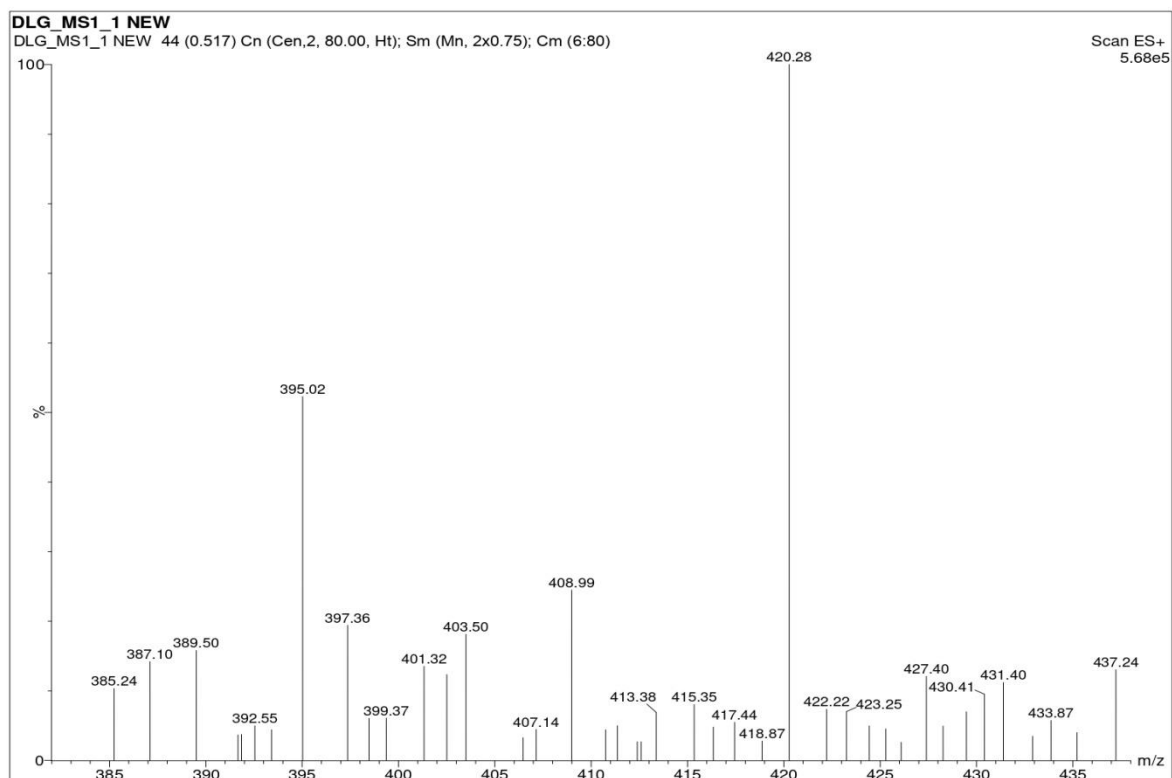


Figure. 3 - Mass spectra of Dolutegravir for precursor MS1

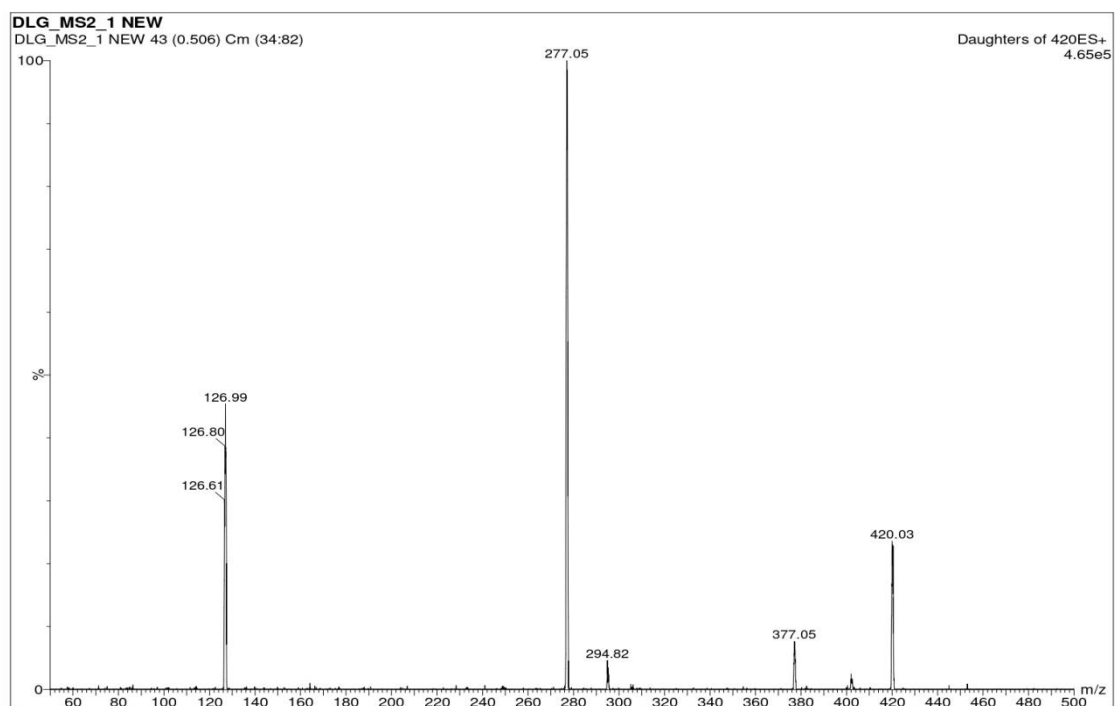


Figure 4: Mass spectra of Dolutegravir product ion masses MS2

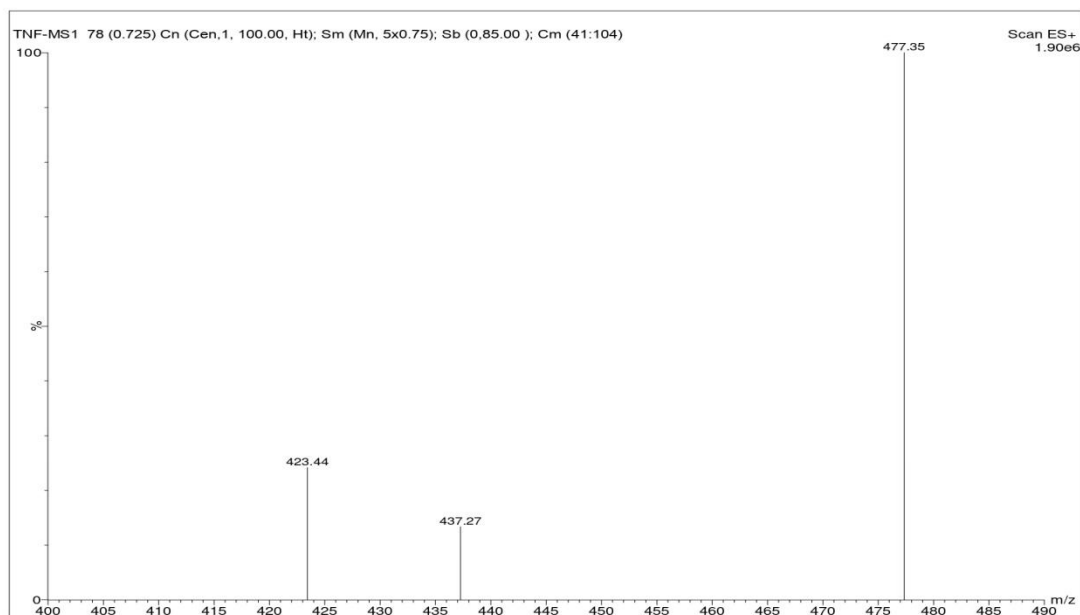


Figure 5 - Mass spectra of Tenofovir for precursor MS1

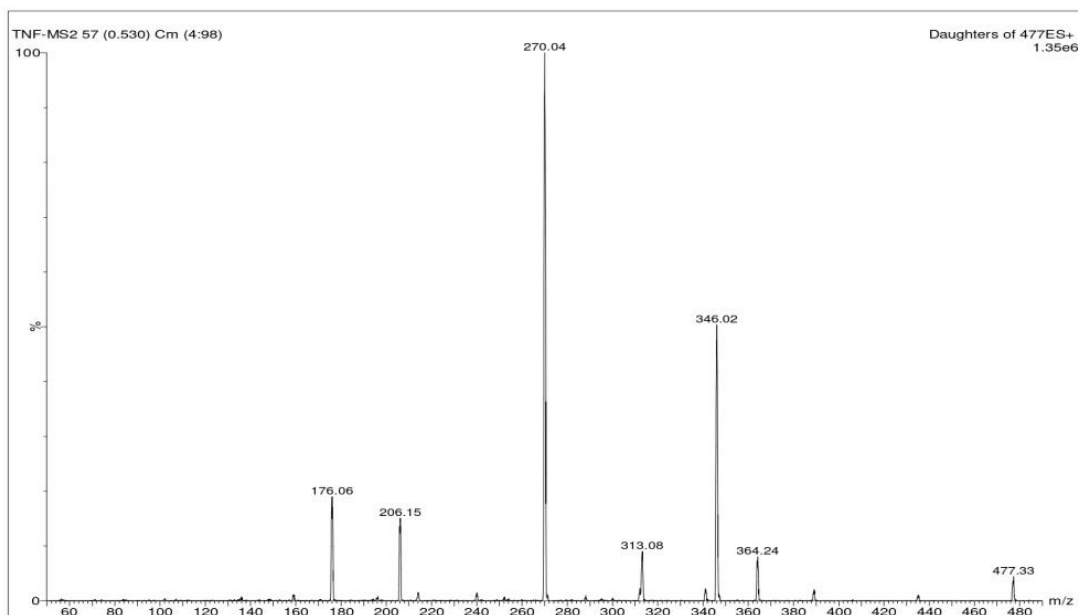


Figure 6: Mass spectra of Tenofovir product ion masses MS2

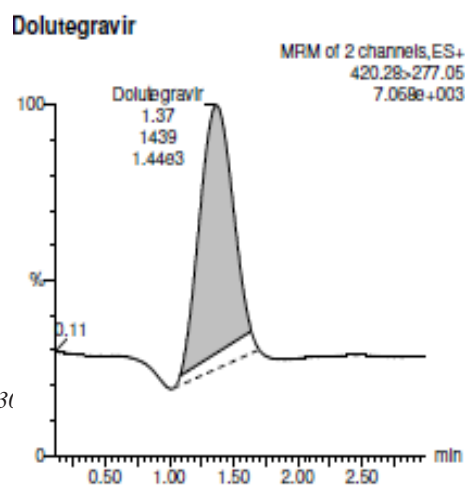


Figure 7 : Chromatogram of Dolutegravir

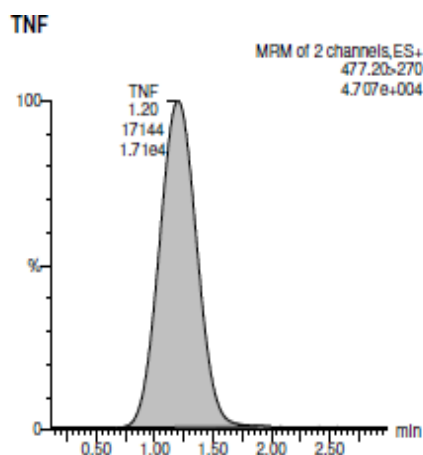


Figure 8 : Chromatogram of Tenofovir

Bioanalytical Method Validation Parameters

Linearity By graphing the peak area ratio vs the concentration (ng/mL), least-squares quadratic regression (LSQR) was used to calibrate the linearity for dolutegravir across a set of 11-point standard curve (2-1000 ng/mL). For every curve obtained, a weighting factor of $1/x^2$ (advised for bioanalytical experiments) was used. x is the proportion of nominal analyte: IS quantity. Through the use of percentage deviation (% DEV), which is the comparative inaccuracy of back computed concentrations of every calibrator, and Pearson correlation (r^2), calibration response functions and linear regression options were examined.

Accuracy and precision Using new sets of the four QC standards—a LLOQ, LQC, MQC, and HQC—accuracy and precision were assessed on four distinct days. In addition to calibration standards obtained freshly every day in duplicate ($n = 8$), all QC and LLOQ standards were made freshly in quintuplet everyday ($n = 20$). Each trial also included an IS plasma control (without analyte) and a dual blank plasma control (without analyte or IS). The accuracy (% DEV) was estimated as the proportion of variation between the nominal quantity and the average of recorded analyte quantity. ANOVA was used to estimate within-run precision (WRP) and between-run precision (BRP), each run day serving as the classifying factor.

Stability Over the course of four hours, the stability of dolutegravir in plasma at ambient temperature was evaluated. After being spiked in plasma, samples (10 ng/mL & 2000 ng/mL) were removed right away ($n = 5$) or by resting at ambient temperature for four hours ($n = 4$). Additional samples ($n=4$) at every concentration was processed with working stocks left at

ambient temperature for 4 hours in order to show the stability of analyte working stock throughout this 4 hour period. For the purpose of comparing analyte and IS quantities, samples analyzed after 4 hours were contrasted with those that had just been produced.

According to FDA Bioanalytical Standards, data collected from auto-sampler, stock solution, freeze/thaw, and benchtop plasma stability were considered satisfactory when the accuracy of measurements varied by no more than 15% at any concentration level.

Extraction efficiency and matrix effects: The suitability of the PPE technique was assessed in six diverse batches of blank matrix through comparison of peak areas of pre- and post-extracted spiked samples from a lowest (2 ng/mL; n = 5) and a highest (1000 ng/mL; n = 5) analyte quantity. By contrasting the peak regions of samples spiked after being extracted (n = 5) in 6 separate sets of blank matrices with samples having equivalent dose spiked in pure mobile phase (n = 5), the matrix effects on the Dolutegravir MS signal were examined. The quantities studied were the same as those mentioned previously, enabling evaluation over the calibration range. By contrasting the peak regions of samples spiked before extraction, processing performance was assessed at lower (2 ng/mL) and higher (1000 ng/mL) concentrations.

Specificity, Selectivity, and carryover Six separate batches of blank matrix were tested for specificity at the lowest calibration concentration (2 ng/mL) to confirm that any internal interference could be distinguished from the peaks of analyte and IS. Chromatographs from spiked and blank matrices were contrasted to assess selectivity. Blank samples were used to test carryover, which was regarded acceptable provided the recorded result did not surpass 20% of LLOQ⁸.

RESULTS AND DISCUSSION

Out of all the tested compositions, a mixture containing ACN and 5 mM AFB with 0.1% FA was demonstrated to be the highly effective mobile phase as well defined and resolved chromatographic peaks were obtained and devoid of tailing. Mobile phase was delivered at 0.120 mL/min and the ions detection was achieved by multiple reaction monitoring as m/z 420.28 to 277.05 for Dolutegravir and m/z 477.20 to 270.00 for Tenofovir. The RT of Dolutegravir and Tenofovir was estimated to be 1.37 & 1.20 min.

Linearity Figure 9 illustrates the calibration curve, which showed linearity of the dolutegravir concentration ranging from 2-1000 ng/mL. The LSQR technique produced a straight line across the dataset that exhibited proportionality constant with little data dispersion. Pearson correlation (r) for dolutegravir was 0.998, as presented in Table 1.

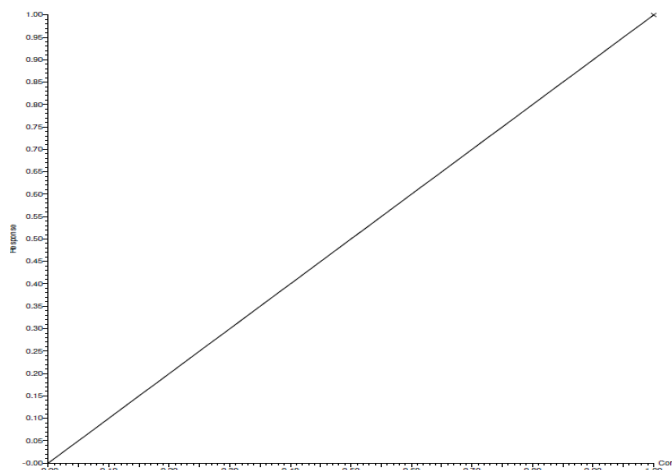


Figure. 9 Calibration curve for Dolutegravir regression analysis

Table 1: Linearity of Dolutegravir

Conc. of Dolutegravir (ng/mL)	Peak: Area (Analyte area/IS area)
2	0.028
4	0.035
15	0.075
50	0.195
200	0.750
500	1.797
800	2.711
1000	3.087

Selectivity

At the Dolutegravir and Tenofovir mass changeover, no discernible interference from internal molecules was seen.

Recovery of the Dolutegravir and Tenofovir

Recovery and mean recovery for Dolutegravir were estimated to fall between 108.9% to 80.3% and 91.46 %, correspondingly. The recovery findings of Dolutegravir is tabulated in Table 2, which shows high extraction yield.

Table 2: Recovery of Dolutegravir from plasma samples

	LQC Response		MQC Response		HQC Response	
	Ext. QC	PostExt. QC	Ext. QC	PostExt. QC	Ext. QC	PostExt. QC
Sample ID	LQC	LQC	MQC	MQC	HQC	HQC
1	648	648	31621	32092	40956	58641
2	710	671	29239	38040	43670	52871
3	832	692	32604	39458	46357	51498
Mean	730	670.3333	31154.67	36530	43661	54336.67
SD	93.61624	22.00757	1730.291	3908.269	2700.511	3790.349
CV%	12.8	3.29	5.55	10.69	6.18	6.97
N	3	3	3	3	3	3
Recovery %	108.9		85.2		80.3	
Overall recovery%	91.46					

QC: Quality Control

Intra-batch precision and accuracy

Intra-batch accuracy ranged for LLOQ QC between 100 % and 100.83 % and for LQC, MQC and HQC varied between 102.01 % to 102.8 %. The findings of intra-batch precision and accuracy for Dolutegravir are assembled in Table 3.

Intra-day precision and accuracy

For LLOQ QC, intra-day accuracy was 80.86 % and for LQC, MQC and HQC varied between 99.21 % to 101.88 %. The findings of intra-day precision and accuracy for Dolutegravir are grouped in Table 4.

Between batch/day precision and accuracy

For LLOQ QC, inter-batch accuracy was 102.48 % and for LQC, MQC and HQC varied between 90.25 % to 115.68 %. The findings of inter batch/inter-day precision and accuracy for Dolutegravir are grouped in Table 5.

Table 3: Intra-batch precision and Accuracy for Dolutegravir

QC samples	Conc. (ng/mL)			
	LLOQ QC	LQC	MQC	HQC
	1	2	480	780
1	2.0	4.37	507.9	683
2	2.37	5.24	491.6	726.8
3	1.87	5.65	506.5	734.4
4	2.34	5.38	498.6	748.4
5	1.76	5.74	486.9	756.3
6	2.26	4.83	502.3	788.2
Mean	2.1	5.20	498.96	739.51
SD	0.25	0.52	8.33	34.96
CV%	12.3	10.0	1.67	4.72
Nominal %	100.8	100	102.8	102.2

Table 4: Intra-day precision and accuracy for Dolutegravir

QC	Conc. (ng/mL)			
	LLOQ QC	LQC	MQC	HQC
	2	5	480	780
1	2.0	4.73	489.9	689.5
2	2.38	5.42	492.6	736.8
3	2.37	5.56	480.5	7354.4
4	2.34	5.83	489.6	784.4

5	2.76	5.47	489.6	765.3
6	2.26	5.83	501.3	789.2
7	2.04	5.37	506.9	788.5
8	2.47	5.24	498.6	762.8
9	1.87	5.52	496.5	743.4
10	1.34	5.48	492.6	749.4
11	2.67	5.64	498.9	753.3
12	2.28	5.83	503.2	788.8
Mean	2.23	5.49	495.02	758.82
SD	0.38	0.31	7.29	28.73
CV%	17.09	5.58	1.47	3.79
Nominal %	80.86	100.42	101.88	99.21
N	12	12	12	12

Table 5: inter batch/day precision and accuracy for Dolutegravir

QC	Conc. (ng/mL)			
	LLOQ QC	LQC	MQC	HQC
	2	5	480	780
1	2.01	4.85	456.9	785.9
2	2.01	4.1	498.7	775.5
3	2.1	4.3	461.6	734.6
4	2.01	4.11	498.7	792.5
5	2.01	4.85	495.9	794.9
6	2.01	4.1	498.6	780.5
7	2.03	4.01	488.4	791.5
8	1.98	4.03	484.4	794.8
9	1.95	4.01	487.4	743.7

10	2.02	4.95	486.5	785.8
11	2.0	4.2	489.4	786.1
12	1.93	4.97	481.9	788.1
Mean	2.004	4.373	486.167	780.439
SD	0.049	0.387	11.367	16.656
CV%	2.4336	8.8392	2.3381	2.1342
Nominal %	102.48	115.68	100.21	90.25
N	12	12	12	12

STABILITY

The stability studies play a very important role in handling and preservation of samples collected, as well as the necessity to retain the integrity of a medicine or at the bare minimum reduce the variance of pre-analysis as low as feasible. Three levels of stability were assessed, including short term stability, long term stability and bench top stability. The international acceptability standards (variation values for area below 15%) were always fulfilled under all conditions, the findings demonstrate that dolutegravir remains stable at the investigated settings.

Bench top stability

Dolutegravir was reported to retain stability up to 6h according to the specified criteria. The nominal% varied between 104.8 % to 107.9 % .Bench top stability findings are tabulated in Table 6.

Long Term stability

Dolutegravir was reported to retain stability up to required time according to the specified criteria. The nominal% varied between 96.2 % to 96.5 % . Results of Long term stability are shown in Table 7.

Short term stability

Dolutegravir was reported to retain stability up to 24h according to the specified criteria. The nominal% varied between 94.5 % to 99.8 % . Results of short term stability are tabulated in Table 8.

Table 6: Bench top stability of Dolutegravir for 6h

S. No.	Conc. (ng/mL)	
	LQC	HQC
	5	780
1	5.54	670.5
2	4.51	511.2
3	5.05	674
4	5.52	678.9
5	4.89	582.9
6	5.28	721.2
Mean	5.13	639.78
SD	0.4	77.5
CV %	7.75	12.12
Nominal %	107.9	104.8
N	6	6

Table 7: Long Term stability of Dolutegravir

S. No.	Conc. (ng/mL)	
	LQC	HQC
	5	780
1	4.75	665.5
2	4.41	680.9
3	5.2	681.5
4	5.28	698.4
5	4.82	710.2
6	5.09	709.5

Mean	4.925	691
SD	0.33	17.93
CV %	6.6	2.6
Nominal %	96.5	96.2
N	6	6

Table 8: Short Term stability of Dolutegravir

S. No.	Concentration (ng/mL)	
	MQC(Fresh)	MQC(Old)
	480	480
1	381.2	304.5
2	368.5	295.7
3	372.8	326.5
4	389.6	315.6
5	401.2	335.6
6	378.4	354.2
Mean	381.95	322.02
SD	11.89	21.36
CV %	3.11	6.63
Nominal %	99.8	94.5
N	6	6

Matrix effect

At low (LQC) and high (HQC) levels of dolutegravir, no detectable matrix impact was seen in any of the tested batches. As indicated in Table 9, dolutegravir's accuracy and precision at LQC level were determined to be 6.69% and 100.9%, while at HQC level, they were 1.59% and 99.29%, correspondingly.

Table 9: Matrix effect of Dolutegravir

Plasma (Batch No.)	LQC (5ng/mL)			Mean	HQC (780 ng/mL)			Mean
	1	2	3		1	2	3	
1	4.34	5.34	4.86	4.85	767.4	726.4	723.5	739
2	4.38	4.34	4.46	4.39	747.9	745.7	727.1	740
3	4.43	4.38	5.34	4.72	727.46	762.8	766.4	752
4	4.32	5.04	4.04	4.47	769.48	758.6	728.7	752
5	4.58	4.84	4.38	4.60	723.5	712.2	757.9	731
6	5.45	5.32	5.23	5.33	767.2	767.4	767.4	767
(Lipemic)	4.95	5.2	5.1	5.07	781.5	738.1	781.5	738.10
(Hemolytic)	4.05	4.97	5.01	5.01	764.9	734.9	781.5	734.90
Mean				4.805	Mean			744.25
SD				0.32	SD			11.86
CV %				6.69	CV %			1.59
Nominal %				100.9	Nominal %			99.29

N	8	N	8
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5.CONCLUSION:

The validated LC-MS/MS technique for quantifying Dolutegravir in human plasma by employing Tenofovir as Internal standard. An effective Protein extraction Technique and isocratic elution on a RP column resulted in an assay suitable for routine analysis. Good separation and no interference of the chromatographic peaks was observed. Utilizing Agilent, Zorbax, XDB C18 (2.1 x 50 mm ID, 5 μ m) plus a mobile phase comprising a combination of 5 mM AFB with 0.1% FA: ACN in 70:30, v/v, supplied at 0.120 mL/min with injecting volume of 10 μ L as well as a run duration of 3 min, an acceptable LC-MS chromatography technique was adopted for quantifying Dolutegravir and findings indicated excellent peak symmetry. Detector used was atmospheric pressure ESI-MS in PI mode (API 4000). The precursor to product ion transitions is m/z 420.28>277.05 for Dolutegravir and m/z 477.20 to 270.00 for Tenofovir were utilized for analysis. The RT of Dolutegravir and Tenofovir were obtained respectively as 1.37 & 1.20 min. The concentration between 2-1000 ng/mL showed linearity with Pearson's coefficient ($r=0.998$) and the total recovery was 92.46 % for Dolutegravir. The CV % findings for both stability studies and accuracy and precision for Dolutegravir were estimated to be below 15 %, which indicate the method to be accurate, precise and stable. The LC-MS/MS approach shown acceptable outcomes with respect to selectivity, accuracy, linearity, sensitivity, recovery, stability, and matrix effect assays. The described approach also provides a benefit of shorter analytic run time than earlier published methods.

REFERENCES

1. "Dolutegravir (Tivicay) Use During Pregnancy". Drugs.com. 16 October 2018. Retrieved 13 February 2020.
2. "Tivicay- dolutegravir sodium tablet, film coated". DailyMed. 24 October 2019. Retrieved 13 February 2020.
3. "Tivicay EPAR". European Medicines Agency (EMA). Retrieved 13 February 2020.
4. "WHO recommends dolutegravir as preferred HIV treatment option in all populations". World Health Organization (Press release). Retrieved 2019-07-22.

5. "ViiV Healthcare announces US FDA approval of the first-ever dispersible tablet formulation of dolutegravir, Tivicay PD, a once-daily treatment for children living with HIV". GSK (Press release). Retrieved 12 June 2020.
6. World Health Organization (2019). World Health Organization model list of essential medicines: 21st list 2019. Geneva: World Health Organization. hdl:10665/325771
7. R.E. Aarnoutse, J.M. Schapiro, C.A. Boucher, Y.A. Hekster, D.M. Burger, Therapeutic drug monitoring: an aid to optimising response to antiretroviral drugs?, *DRUGS*, 63 (2003) 741- 753.
8. Pauline DJ t al, Development and Validation of UPLC-MS/MS method for Qunatification of Antiretroviral Drugs in Human Plasma, *Journal of Chromatography B*, 2018.