RESEARCH ARTICLE

Development and Validation of UPLC-MS/MS Bioanalytical Method for Simultaneous Quantification of the Antiretroviral Drugs Dolutegravir, Lamivudine, and Tenofovir in Human Plasma

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ABSTRACT

The current proposed validated bio-analytical UPLC-MS/MS method for assessing components like dolutegravir, lamivudine, and tenofovir in human plasma using bictigravir as an internal standard with a mobile phase of formic acid (0.1%), 2 Mm ammonium formate in water and acetonitrile (30:70, v/v), and optimized flow rate of 0.5 mL/min, the separation was performed on an Agilent XDB C18 column (250 mm X 4.6, 5µ). The three components were analyzed by using LC-MS/MS in positive ion mode owing to the presence of primary groups. For the technique validation for the concentration range of 20 to 1000ng/mL for dolutegravir, lamivudine, and tenofovir in human plasma, bitigravir was employed as the internal control. The three-drug moieties average recoveries from spiking plasma samples were discovered to be reproducible. Based on the data displayed above, it was determined that the procedure was rapid and dependable, with a minimum total run duration of 3.0 minutes. The stability studies were assessed as a consequence of the current approach's successful validation in accordance with the food and drug administration (FDA), european medicines agency (EMA), and international council for harmonisation (ICH) requirements.

Keywords: Dolutegravir, Lamivudine, Tenofovir, Bictigravir, Ultra-performance liquid chromatography-mass spectrometry, Internal standard method, Human plasma.

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Conflict of interest: None

INTRODUCTION

Dolutegravir is used for the human immunodeficiency virus (HIV) Integrase inhibitor oxazine-9-carboxamide (Figure 1) that hinders strand transfer, hence blocking the integration of retroviral DNA into the host cell (MASUDA and KATO, 2020). Another, antiviral medication; lamivudine 4-amino-1-[(2R, 5S)-2-(hydroxymethyl)-1, 3-oxathiolan-5-yl]-1, 2-dihydropyrimidin-2-one (Figure.1) is used to treat and prevent HIV/AIDS (Figure. 1) (Margolis *et al*., 2014). It functions by impeding the enzyme nucleotide reverse transcriptase. Tenofovir alafenamide, isopropyl (2S)-2-[[(1R)-2-(6-aminopurin-9-yl-1-methyl-ethoxy] methyl-phenoxy-phoshoryl] amino] propanoate, are used to treat adults with HIV/AIDS (Figure 1) through nucleotide reverse transcriptase enzyme inhibition activity (Atkinson and Petrozzino, 2009). In a fixed-dose regimen, dolutegravir, lamivudine, and tenofovir are given as second-line treatment (Naveen *et al*., 2013).

The completed literature review[1-5] results showed very few liquid chromatography tandem mass spectrometry (LC-MS/MS) techniques[6-12] for the drugs described above in human plasma (Grande *et al*., 2019). The current investigation was found to take less time and economic as a consequence. Table 1 includes a detailed description and comparison of the proposed method with an existing method.

MATERIALS AND METHODS

Chemicals and Reagents

Bictegravir, dolutegravir, lamivudine, and tenofovir were purchased from Hetero Labs in Hyderabad, Telangana, India. K2 EDTA human plasma, Milli-Q Water, acetonitrile, and methanol are all HPLC-grade products procured from Merck, Rankem, and Rankem, respectively.

Instruments for Developing and Validating Methods

The Make and Model of the equipment utilized for the present research study are detailed in Table 2.

LC-MS/MS Assay Condition and Details of Equipment

The UPLC agilent (1290) system was used, which included column ovens (CTO-AS), degassers (DGU-20 A3), solvent supply pumps (LC-20AD), and other parts. The high-

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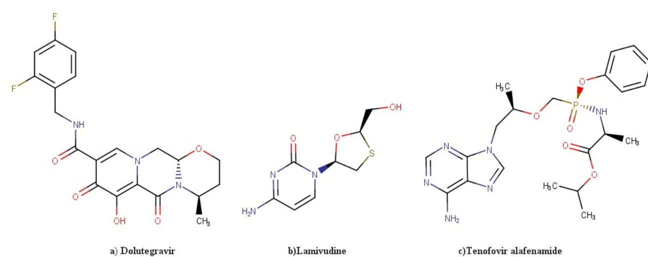

Figure 1: Tenofovir alafenamide, lamivudine, and dolutegravir chemical structures

performance auto-sampler (SIL HTC) was also used. A three-part apparatus (Mass Analyzer - Waters - Quattro Premier XE) was utilized for mass spectrometric detection in the multi-power reaction monitoring (MRM) mode. Data processing has been done using Mass Lynx, version 4.1, and the positive ionization interface.

Prerequisites for Mass Spectroscopy

Mass spectrometry is based on the creation and identification of ions divided by their mass-to-charge ratio (m/z). By perusing a mass spectrometer, the main component parent ion and any fragmented ions related to it were analyzed. A 70:30 mixture of acetonitrile and formic acid 0.1% v/v was used to prepare 500 ng/mL analyte solutions and internal standards, which were subsequently administered using a 10L injection volume. To solve each component's/internal standard. For every 100 to 600 atomic mass units, each analyte and its corresponding internal standard were measured and reported. The product ions are obtained through additional scanning in MS/MS mode once the parent ion has been found. The resolution was calculated in mass units, with nitrogen as the collision gas and zero air as the sheath gas. According to Table 3 and Figure 2 and 3, the fragment ion with the highest intensity was selected for multiple reaction monitoring (MRM).

Optimization/Tuning of the Mass Spectrometry

The optimization/tuning of the three components based on their molecular weights identified the parent and daughter ions of each analyte precisely. The three analytes are, therefore, polar and ionizable (Rigo-Bonnin *et al*., 2020). As a result, the investigation was conducted using the electron spray ionization (ESI) method. After determining that the parent could deliver acceptable values, the composite and gas properties were optimized at a 0.15 mL/min flow rate. A "T" connector was used to attach the syringe pump to the detector, and the LC pump and LC pump were connected to one another. The 'Positive ionization mode' was employed for the liquid chromatography that was used to identify the ions.

Optimized Conditions for Mass-Spectrophotometric Method

Table 4 summarizes the chromatographic and mass spectrometric settings, and the (MS) mass spectra of the 3 components lamivudine, tenofovir and dolutegravir.

Table 1: Summarized the validated parameter(s) used in old & new methodologies.

S. No.	*Parameter*	*Reported method (Raju et al.)21*	*Current method*
1	Column	Column made of Zorbax Coulmn C18 (150 cm x 4.6 mm, 5 m)	(2.1 X 50 mm, 5.5 m) Zorbax XDB C18 Column
2	Mobile Phase	Water (85:15%) with 0.1% formic acid in methanol	ACN: H2O (70:30) and Formic acid (0.1%)
3	Flow Rate	1.0 mL/min.	0.15 mL/min
4	Run Time	4.0 minutes	3.0 minutes
5	The peak period	Compared to more	Comparatively less
6	Internal Standard	For each of the three different medications, three internal standards were applied.	For all three medications, a single internal standard is utilized.

Optimization of the Extraction Procedure

Protein precipitation is a technique for reducing matrix interference with the analyte. Organic acids were used as precipitants. Various organic solvents, including ethanol, methanol, isopropanol, acetonitrile and acetone, are frequently utilized to eliminate plasma proteins and improve desirable with contemporary HPLC technology (Tsiang *et al*., 2016). The precipitating agent is used to dilute and vortex the sample matrix. Following centrifugation, the bulk proteins are removed through filtration. The interested analyst promptly evaluates the resulting supernatant liquid or filtration. The "protein precipitation technique" requires the analyte to be freely soluble in the reconstituted solvent.

Sample Extraction Procedure

As a result, the frozen plasma samples were removed from the freezer and subjected to the following method. Each 100 µL aliquot of the sample was held in a 5 mL polypropylene tube. After that, 300 µL of acetonitrile and 20 µL of ISTD solution (1 g/mL Bictigravir) were used to precipitate it (Van Lunzen *et al*., 2012). The components of the mixture were vortexed to blend them before being precipitated and all the samples were injected into the UPLC/MS/MS.

Optimized of Chromatographic Condition

Determine the optimal chromatographic conditions by analyzing the standard solutions of three analytes. The numerous experiments that were conducted are listed in Table 5. Trial-V was determined to be the optimal condition based on the results of the aforementioned Chromatographic trials, so the current bio-analytical method was developed and validated using the same experimental parameters as per ICH guidelines.

Mobile Phase Preparation

Preparation of 0.1 v/v formic acid

Pipetted 1-mL of formic acid into 1000 mL of UPLC/UFLC-compatible double-distilled water. After 10 minutes of

Table 2: Instruments for developing and validating methods

S. No	*Instrument*	*Model and manufacture*
1	Mass spectrophotometer	Agilent , Mass Lynx, Ver. 4.1, Quattro Premier XE
2	UPLC	The Acquity UPLC WATERS
3	Fully automatic digital balance	AXIS ACN 220G
4	Deep freezer	Blue star 100
5	Microcentrifuge system	Apotex, Medico Plus
6	Cyclo mixers	CM101 (Lab India)
7	Micropipettes	Apex scientific

Table 3: Conditions for mass reaction monitoring (MRM)

MRM conditions	*Value*
Temperature at source	120°C
Temperature of desolvation	400°C
Capillary volts.	4.00
Gas desolation (Liter per hour)	850.00
(L/hr) cone gas	100.00
Cone.	37
Energy of collision	Bictegravir – 24, Dolutegravir-24,Tenofovir– 31, Lmivudine-11

ultrasonic degassing, A membrane filter with a 0.45-micron opening was used to filter the mixture.

Preparation of mobile phase

Under vacuum filtration, a precise mixture of 600 mL (60%) of formic acid (0.1%) and 400 mL l (40%) of acetonitrile was filtered using 0.45-micron membrane filter.

Preparation of diluent

Preparation of diluents composition of 50:50 of methanol and double distilled water were mixed and measured. After diluents were filtered using 0.45-micron membrane filter paper.

Needle wash and seal wash solutions

Needle and seal wash solutions are made from diluent containing 50% methanol.

Preparation of internal standard solution

Acetonitrile and water were combined in a 50:50 v/v ratio to create the bictigravir with a standard internal concentration of 1 g/mL.

Preparation of standard stock solution

In 10 mg of dolutegravir, tenofovir, and lamivudine were added to a 10 mL volumetric flask, and 7 mg of the composition of mobile phase (MeoH: Double distilled water, 50:50%) to create a standard stock solution (Veerareddy *et al*., 2021). Following thorough shaking, the volume was adjusted with diluent to produce a 1000 g/mL concentration for each medication.

Preparation of test (sample) solution

In 20 tablets containing the equivalent of 10 mg of TNF were crushed, diluted in diluent, and then run through a 0.45-micron filter. The concentration of the sample solution was diluted further using the mobile phase solvent until it reached 10 ng/mL.

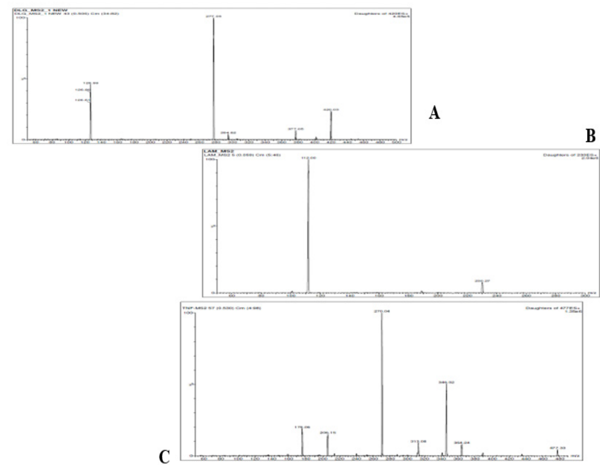

Figure 2: Dolutegravir, Lamivudine, and Tenofovir product ion mass spectra (A), (B), and (C), respectively

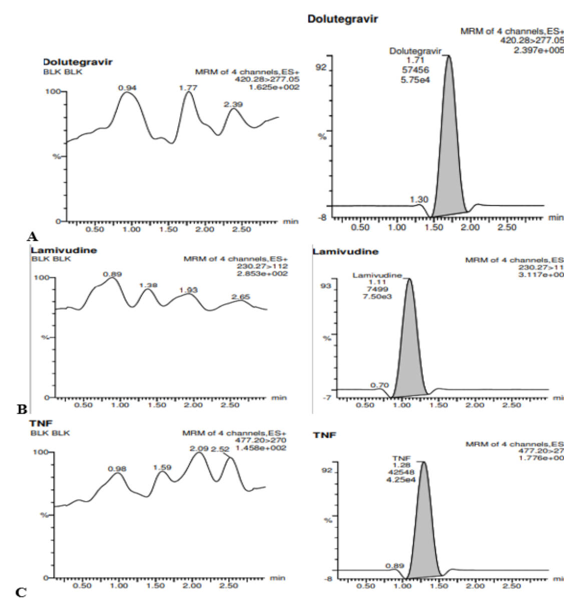

Figure 3: MRM Chromatograms of Tenofovir (right panel), Dolutegravir (left panel), and Lamivudine (right panel) as well as a blank (left panel).

Mixed standard solution preparation

Dolutegravir, tenofovir, and lamivudine (Mixed Stock solution) were accurately weighed, and 1-mg of each analyte was dissolved in 1-mL of diluent using vortexing.

Preparation of workable solutions

Aliquot a 20 mL stock solution of each of the aforementioned solutions into a 2 mL of solvent (diluent) that contains 10.00 Mcg/mL of each analyte (Kashid and Kadam, 2021). Transfer each of the 100 L solutions described above to a 2.0 mL diluent containing 500 nm/mL of each analyte. The information was utilized in a number of ways to modify the mass spectrophotometric monitoring parameters. To generate solutions with a concentration of 100 ng/mL, 20 L of each

Table 4: The optimized conditions for mass-spectrophotometric method

UFLC	*Agilent UFLC system*
Mass	Triple quattro mass analyser
Polarity	Positive ion mode
Ion source	Electron ion Spray
Detection ions	
Dolutergravir	420.28 amu (Parent), 277.05 amu (Daughter)
Tenofovir	477.20 amu (Parent), 270.00 amu (Daughter)
Lamivudine	230.27 amu (Parent), 112.00 amu (Daughter)
Bictegravir (Internal Standard)	450.10 amu (Parent), 289.00 amu (Daughter)
Column	Waters C_{18} 250mm X4.6milli meter, 5μicron
Optimized column temperature	32°C
Mobile Phase	10 mM Phosphate Buffer P^H 2.8:Acetonitrile (80:20 v/v)
Flow Rate	1.00 mL/min
Volume of injection	20 μL
Retention Time	Dolutegravir–1.71 min Tenofovir–1.28 min Lamivudine–1.09 min Bictegravir (Internal Standard)–1.82 min

Table 5: the specifics of the trials that were conducted before the technique parameters were optimized.

Trial No.	*Conditions*	*Remarks*
1st Trial	0.25 mL/min flow rate, Accuity UpLC Beh column ACN:0.1% Methanol:70:30 Formic acid, Blank: C18 2.1X50 mm, 1.7 m Diluent	A Peak shape was not good.
2nd Trial-	Agilent Zorbax XDB C18 2.150 mm, 5, Diluent: 50% Methanol, ACN:0.1% Formic acid (80:20), 0.15 mL per minute is the flow rate. Blank Diluent	Asymmetric peaks were observed.
3rd Trial	2.1 mm, 50 mm, 5 mm, ACN:0.1% Formic acid (80:20), Agilent Zorbax XDB C18 50% Methanol as a Diluent 0.20 mL/min, flow rate Blank Diluent	The peak resolution was not good
4th Trial	Agilent Zorbax XDB C18, 70:30 ACN:0.1% Formic acid, 2.1 mm, 50 mm, 50% Methanol as a Diluent 0.20 mL/min, flow rate Blank Diluent	Peak resolution was low
5th Trial-(Optimized Condition)	Diluent: 50% Methanol and 70% ACN:0.1% Formic acid. Agilent Zorbax XDB C18 2.1X50 mm, 0.15 mL/min is the flow rate. Blank Diluent	Observed good shape and sharp peaks obtained.

solution with a concentration of 10 g/mL was extracted and 2 mL of diluent was added. At room temperature, daily dilutions were performed on the working solutions generated during the analysis (Hassounah *et al*. 2017).

The process of preparing plasma-spiked calibration of quality control samples and standards

To construct the "calibration standards" and "quality control (QC) samples," "blank plasma" was spiked with recently invented functional standard solutions. Before, blank plasma batches were combined. usage after being received from healthy, pre-screened individuals (Neogi *et al*., 2018)raltegravir (RAL. Calibration standards for dolutegravir, tenofovir, and lamivudine were produced at concentrations of 2, 4, 10, 50, 100, and 3.7-250, 500, 800, and 1000 ng/mL. Quality control samples measuring 5 ng/mL at LQC, 480 ng/mL at MQC, and 780 ng/mL at HQC were prepared for each drug.

Procedure for sample extraction optimization

For the intended mass spectrophotometric analysis, the protein precipitation extraction process was chosen to be refined to produce consistent, reproducible results with minimal matrix effects. Each analyte sample received 950 μL of human plasma before being vortexed. After adding 1-mL of the mixture was vortexed for 5 minutes, followed by 10 minutes of centrifugation, using ACN. To use the LC-MS/MS apparatus, extraction of 0.8 mL supernatant solution from the final mixture, was transferred to sample vials, and utilized.

System suitability criteria and mobile phase

During the phase of technological development, the composition of the mobile phase was decided. For the validation and research phases, a significant amount of mobile phase with consistent results may be created by mixing 700 mL of acetonitrile with three hundred (300 mL) of formic acid at a concentration of 0.1%. Throughout the validation studies, a system suitability test (SST) was conducted to ascertain whether or not the system's performance was adequate for the study. Six identical system suitability solutions were generated and injected as part of the SST test. Prior to the analysis, the effectiveness of the instrument was evaluated (Kokkirala and Suryakala, 2019).

Method Development

Methodological development begins with scanning parent and fragment ion analyte solutions utilizing 200 ng/mL dolutegravir, tenofovir and lamivudine as analytes, and bictigravir (BCT) as an Internal standard. The analyte solution was injected, and the parent weight of the analyte was scanned. In addition, the parent ion was examined for the MS/MS mode product ions. The protons have been adjusted into positive mode utilizing electro-sprinkler technology based on analytes and ISTD's capacity to receive them. Scanning in the range between 100 and 600 amu has been carried out. Their polarity and the presence in their structures of highly ionized functional groups like the amine or carboxylic acid have great sensitivity to antiviral products in mass ionization analyses compared to non-polar and ionizable organics all parent peaks and daughter ion peaks were eluted appropriately. In optimizing compound parameters for all three analytes, BCT used comparatively high collision energy to get an appropriate response and the respective Mass chromatograms were detailed in following Figure 4.

Method Validation

The validated technique for concurrently assessing DLG, LMN, and TNF was created using ICH guidelines. Finding new

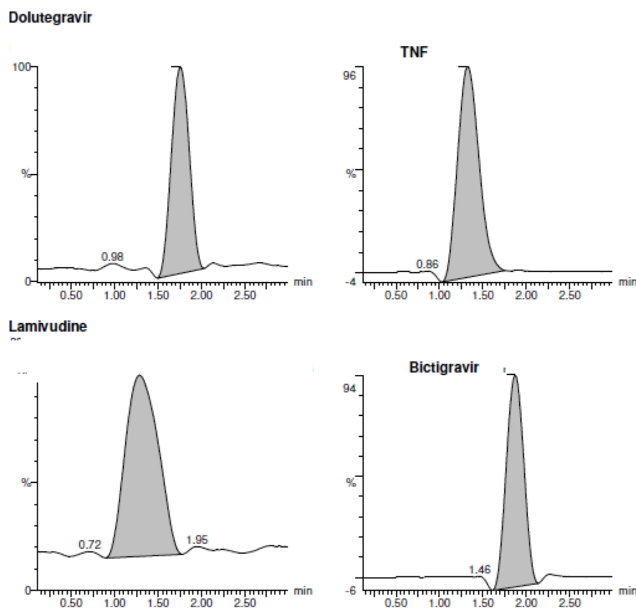

Figure 4: Chromatograms of dolutegravir, tenofovir and lamivudine

drugs requires the development and approval of bioanalytical techniques. The method validation includes numerous parameters. In addition, a stability study of the prescribed medications was conducted and evaluated. The current study includes analyses of accuracy, precision, recovery, matrix factor, stability, specificity, sensitivity, and selectivity. It also contains fresh quality control (QC) samples, lower and upper quantification limits (LLoQ and ULoQ), and calibration curves, and linearity range.

Specificity

At an LLoQ concentration of 2 ng/mL, three replicates of one Blank containing 200 L of human plasma were examined.

Selectivity

This concept refers to the capacity to measure an analysis technique's selectivity and identify endogenous matrix components such as metabolites, pollutants, matrix elements, breakdown products, and so on. The selectivity of the procedure was studied and evaluated using the internal standard (ISTD), blank plasma, LLoQ sample, and blank plasma.

Procedure

Sample vials for lamivudine, dolutegravir, tenofovir, blank, and Blank + ISTD were made. In 10 mL of 1-mg/mL aqueous solutions were diluted with 2 mL of diluent to produce a 5 g/mL concentration. In 950 mL of plasma was added to 50 mL of each combination. After combining the final solution with the internal standard (ISTD), 200 liters of it were vortexed.

Calibration curve

Analyzing sample solutions in the same biological matrix that will be used for research and injecting the biological matrix with known analyte concentrations can provide calibration curves. Calculate the concentrations of each standard using the concentration range.

Table 6: Linearity data (parameters of the regression equation)

Parameters	*Dolutegravir*	*Lamivudine*	*Tenofovir*
Range (ng/mL)	2-1000	2-1000	2-1000
R^2	0.999	0.998	0.997
Slope	0.0021	0.00029	0.00153
Y-intercept	0.1075	0.1543	0.0305

Procedure

The linear range is calculated as the minimum concentration between five and eight. The blank and zero samples are not included in the linearity. The linearity of each calibration curve could be ascertained by comparing the peak area ratio of the analytes' 'y-axis' to their 'x-axis' nominal concentrations. The calibration curves for dolutegravir, tenofovir, and lamivudine (presented in Table 6) were found to be linear, with an R-value of more than 0.99 between 2 and 1000 ng/mL.

Range of quantification

According to the concentration response term "Quantification Range" refers to the concentration range. Calculating the linear range using the lowest concentration between five and eight. The zero and blank samples are excluded from the linearity calculation. By plotting the ratio of peak areas of the analyte's 'y-axis' against their 'x-axis' nominal concentrations, researchers could assess the linearity of each calibration curve. Dolutegravir, tenofovir, and lamivudine calibration curves (shown in Table 6) were linear with R2 values more than 0.99 between 2 and 1000 ng/mL.

Fresh QC samples

To the assessing of accuracy and stability of analytes, use the most current quality control samples (QCs). Analysis stability and method performance are set with the help of QCs. Performance quality checks are offered to evaluate the accuracy and correctness of the technique.

Accuracy

A bioanalytical approach's 'exactitude' can be evaluated by contrasting the test findings with the analyte's nominal concentration. The "Accuracy" parameter assesses the precision of samples for which the analyte concentrations are known. It determines how closely the test results obtained using that approach matched the actual value. A minimum of three levels and five concentration measurements should be employed to test accuracy on samples spiked with the analyte in known proportions. The percent variance coefficient (% CV) is used to assess the precision of the developed method.

Matrix factor

When sample quantities are assessed, the "Effect of Matrix" refers to the cumulative matrix effect that includes every element of the sample solutions except for the individual analytes. "Matrix Factor" denotes the strength of the "Matrix Effect." Co-eluting matrix constituents may impact and modify the ionization process. However, any measurable response in matrix blanks might not have an impact on the accuracy and

Table 7: The Dolutegravir, tenofovir, and lamivudine (Bench-Top and Long-Term) stability sample preparation procedures.

Conc. of Stock in μg/mL	*Volume of stock in mL*	*Volume of plasma in mL*	*Final Vol. in mL*	*Final Con. in ng/mL*	*Content details*
50 (Stock old)	0.050	0.951	1.0	5.0	LQC (Old)
0.50 (Fresh stock)	0.050	0.950	1.0	5.0	LQC(Fresh)
0.780 (old stock)	0.050	0.950	1.0	780	HQC(Old.)
0.780(Stock solution Fresh)	0.050	0.950	1.0	780	HQC(Fresh)

Table 8: Details of Short-term Stability Study of samples

Preparation(s) –Dolutegravir, Tenofovir and Lamivudine Short-term Stability Study					
Conc. of Stock (μg/mL)	*Stock Volume (mL)*	*Diluent Volume (mL)*	*Final Volume (mL)*	*Final Concentration (ng per mL)*	*Details*
0.480 (stock of old)	0.050	0.950	1.0	480	MQC (Old stock)
0.480 (stock of fresh)	0.050	0.950	1.0	480	MQC (Fresh stock)

precision of the approach because MS selectivity detection depends on detectable reactions in matrix blanks. "Matrix Effect" also refers to the quantitative analysis of matrix effects caused by ionization enhancement or reduction in a mass spectrometric detector.

Stability

It is possible to check the chemical or physical compatibility of "stability" in a given matrix under precise conditions and at particular times. To assess the matrix effect, blank plasma was obtained from three distinct sources, including a lipemic and a hemolytic lot. After extraction, a mobile phase containing predefined analyte concentrations was reconstituted and contrasted with the aqueous samples. LQC level and ISTD samples were also taken. The matrix factor for analyte/ISTD was used to compare the peak response when matrix ions were present and when they weren't. The mean of the aqueous samples is contrasted with each response ratio from the batch of post-extracted matrix samples.

Statistical Analysis

The relevant samples were examined under several stability settings, including benchtop, long-term, and short-term stability, and the findings of the stability computation were made. As a result, the stability of each analyte may be analyzed using the findings of the stability research and the preparation information contained in Tables 7, 8, and 9. The outcomes were generated using the "Mass Lynx software" (Version 4.1), and they were expressed as "Mean" with "Standard deviation," "%RSD," and "Coefficient of regression" for the variables of accuracy, precision, and linearity, respectively.

Table 9: Data of Calibration curve

Analyte	*Concentration (nominal)(ng/mL)*	*Concentration(Typical) (ng/mL)*
DLG	2	1.810
	4	3.919
	10	10.496
	50	50.459
	100	107.385
	500	49.284
	800	782.027
	1000	1014.620
TNF	2	1.930
	4	3.976
	10	13.177
	50	56.131
	100	88.413
	500	520.708
	800	831.723
	1000	95.118
LMN	2	2.197
	4	3.444
	10	11.380
	50	28.425
	100	9.914
	500	224.904
	800	83.442
	1000	1020.207

RESULTS & DISCUSSION

System Suitability Studies

The "specifications" are viewed as a measurement derived from the analysis to ensure optimal system performance for the study. The term "Specific and Sensitive" describes the proposed method's level of precision and thoroughness in eluting the analyte peaks.

Selectivity of analytes

Because excipients that are frequently present did not interfere with the elution of the analyte peaks of interest, the method is known as "Selective."

Calibration curve

A matrix-based calibration curve pertaining to the three topical metabolites was used to determine the analyte concentration in the tested unknown samples. Each correlation coefficient (R2) of the dolutegravir, tenofovir, and lamivudine calibrating curves between 2 and 1000 ng/mL was found to be more than 0.9994, indicating that they are all linear. Tabulated results were shown on Table 9.

Table 10: Precision data

Drug	*Concentration (ng/mL)*	*Peak area*	*%RSD*	*Retention time (min)*	*%RSD*
Dolute gravir	10	3146.15	1.22	2.37	0
		3193.69		2.37	
		3172.89		2.37	
		3235.92		2.37	
		3162.75		2.37	
		3240.84		2.37	
Lamiv udine	10	1572.81	1.29	0.85	0
		1537.51		0.85	
		1564.59		0.85	
		1586.23		0.85	
		1567.02		0.85	
		1596.60		0.85	
Teno fovir	10	3813.04	1.20	1.16	0
		3780.82		1.16	
		3837.66		1.16	
		3735.32		1.16	
		3804.49		1.16	
		3868.32		1.16	

Quality control of sample(s)

Three separate levels of concentration (one at 3.0 X LLOQ, one in the middle, and one at the top of the range) of fresh quality control (QC) samples were combined.

Precision and accuracy studies

Both the lower and upper limits of quantification limits, as shown in Table 10, should be established as LoQ with adequate precision and accuracy.

Extraction of recovery studies

Recovery is the difference between a detector's response at a precise, pure standard concentration and a detector's response that depends on how much analyte is added to and removed from the matrix. Pre-extraction plasma samples (n=3) were compared to extraction plasma samples to evaluate the amount of lamivudine (at low, medium, and high concentrations) and dolutegravir (at low, medium, and high concentrations).

Matrix effect

The co-eluting matrix's components can alter ionization for the better or worse, but they might not significantly change the result. Several lots are used to evaluate the matrix effect. The evaluation of the response ratio between each post-extracted matrix lot and its aqueous counterpart. By comparing the peak response of the matrix ions present with that of the absence, the matrix factor for the analyte or the ISTD was derived.

Stability studies

According to the necessary experimental parameters and under certain stability conditions, the stability of the three analytes was evaluated, and the percentage stability was derived as a consequence.

Benchtop stability

Benchtop (BT) stability was investigated to prevent analyte deterioration during the analysis or extraction of experimental samples. Six quality control samples were thawed to room temperature (about 25°C) after being taken out of the freezer. Comparable samples from the benchmarks for sample quality and stability control were processed and assessed after six hours. To offer stability, the stability concentration and comparison samples were evaluated and computed.

Long-term stability studies

To prevent analyte degradation during sample analysis or extraction, benchtop (BT) stability was evaluated. Six quality control samples were removed from the freezer and brought to room temperature (roughly 25°C). Six hours later, samples comparable to the sample quality and stability control standards were processed and evaluated. The stability concentration and comparison samples were analyzed and computed to ascertain the stability percentage.

Short-term stability studies

Between sample collection and analysis, shorter storage stabilities were investigated to demonstrate analyte stability within the test system matrix. After the models were stored at room temperature in a laboratory environment (aqueous comparison samples), three duplicates of each component were fabricated and analyzed using newly-established samples used for quality control and calibration standards. By comparing the average concentration of stability samples to the average concentration of samples, the percentage of stability was calculated.

CONCLUSION

It is discovered that the three model pharmaceuticals (dolutegravir, tenofovir, and lamivudine) can be selectively detected by a newly developed bio-analytical technique with a reasonable degree of specificity. Creation, the current bio-analytical approach was validated stability study was formulated in agreement with FDA, EMA, and ICH recommendations, and acceptable standards were verified. The study makes use of affordable solvents, and it has been shown that the outcomes are repeatable. There were no detected pollutants or interferences. Compared to earlier techniques, the medicines under investigation are efficiently analyzed and obtained with greater resolution and shorter retention times. The "Accuracy," "Precision," and "%Recovery" validation variable findings satisfied the "Linearity" requirement. Furthermore, it was shown that the new approach reduced the matrix effect. The stability investigations were likewise deemed satisfactory following the computation of the attained percentage of stability. Using LC-MS/MS technology, the proposed bio-analytical method can simultaneously quantify the selected anti-retroviral fixed-dose regimen (Dolutegravir, Tenofovir, and Lamivudine).

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