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Ensuring Stability and Accuracy: Bioanalytical Validation of Elexacaftor, Ivacaftor, and Tezacaftor in Human Plasma by HPLC Analysis

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ABSTRACT

This study presents the stability-indicating bio analytical validation of Elexacaftor, Ivacaftor, and Tezacaftor in human plasma using HPLC. The method employs reverse-phase chromatography to ensure accuracy and precision in quantification. Chromatographic conditions involve a stationary phase of Azilent (250 x 4.6 mm, 5 m), with a mobile phase comprising 0.01N Potassium di-hydrogen phosphate (pH: 3.5) and Acetonitrile in a 70:30 (v/v) ratio. The flow rate is maintained at 1.0 ml/min, with detection at a wavelength of 250nm and a column temperature set at 30°C. Lumacaftor serves as the internal standard. Retention times for Ivacaftor, Elexacaftor, and Tezacaftor are determined to be 2.391 min, 3.208 min, and 3.644 min, respectively. The %CV for Elexacaftor, Ivacaftor, and Tezacaftor are 0.08%, 1.05%, and 3.59%, respectively, while recovery rates are found to be 96.41%, 95.029%, and 98.21%. The method exhibits excellent linearity over concentration ranges of 435-17400 ng/mL for Elexacaftor, 60-2400 ng/mL for Ivacaftor, and 300-1200 ng/mL for Tezacaftor (r2 = 0.999). Lower limits of quantification are established at 435 ng/mL for Elexacaftor, 600 ng/mL for Ivacaftor, and 300 ng/mL for Tezacaftor. Validation according to ICH guidelines confirms the method's suitability for pharmacokinetic and therapeutic drug monitoring. Overall, this stability indicating HPLC method provides a robust approach for the accurate quantification of Elexacaftor, Ivacaftor, and Tezacaftor in human plasma.

KEY WORDS: Elexacaftor, Ivacaftor and Tezacaftor; RP-HPLC

INTRODUCTION[1-7]

Elexacaftor is a compound characterized by the chemical structure N-(1,3-dimethylpyrazol-4-yl) sulfonyl-6-[3-(3,3,3-trifluoro-2,2-dimethylpropoxy) pyrazol-1-yl]-2-[(4S)-2,2,4 trimethylpyrrolidin-1-yl] pyridine-3-carboxamide. Tezacaftor is defined by the molecular structure 1-(2,2-difluoro-1,3-benzodioxol-5-yl)-N-[1-[(2R)-2,3-dihydroxypropyl]-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl) indol-5-yl] cyclopropane-1-carboxamide. Ivacaftor, on the other hand, is identified as a N-(2,4-ditert-butyl-5-hydroxyphenyl)-4-oxo-1H-quinoline-3-carboxamide medication utilized for managing Cystic Fibrosis (CF) in patients aged 2 years and older.

LITERATURE REVIEW [8-13]

Several techniques have been documented in the literature for quantifying Elexacaftor, Tezacaftor, and Ivacaftor in human plasma. However, based on a comprehensive literature review, no method utilizing RP-HPLC for the estimation of these compounds in human plasma has been reported.

MATERIALS [14-25]

Elexacaftor, Tezacaftor and Ivacaftor**,** Distilled water, Acetonitrile, Phosphate buffer, Methanol,sodium dihydrogen phosphate, tri ethylamine, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem

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INSTRUMENTS

s.no	Instrument	Company name	Brand name
1	Electronic balance	Sartorious	Denver
2	pH meter	Metsar	BVK enterprises
3	Sonicator	Lab man	BVK enterprises
4	Centrifuge	Thermo Fisher	-
5	Vertex	Remi CM101	-
6	HPLC water	Alliance	Water HPLC 2695 SYSTEM

METHODS:

Buffer: Add 7.0 mL of triethylamine into a 1000-mL flask containing 900 mL of water. Adjust the solution with phosphoric acid to a pH of 3.0 ± 0.1. Dilute with water to volume and mix well.

Mobile phase: Acetonitrile, and Buffer (30:70)

Preparation of Elexacaftor Stock solution (870 µg/ml):

Take 87 mg of Elexacaftor in 100 ml volumetric flask and make the volume with diluent to produce 870 µg/ml.

Preparation of Elexacaftor Spiking Solutions:

From the above Elexacaftor 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 10 ml volumetric flask and make up the volume up to the mark with diluent to produce 4.35 µg/ml, 8.70 µg/ml, 13.05µg/ml, 34.80 µg/ml, 87.0 µg/ml, 104.4 µg/ml, 139.20 µg/ml and 174.0 µg/ml. Calibration standards and quality control (QC) samples were prepared by spiking blank plasma with working stock dilutions of analytes to produce 445 ng/ml, 870 ng/ml, 1305 ng/ml, 3480 ng/ml, 8700 ng/ml, 10440 ng/ml, 13920 ng/ml and 17400 ng/ml.

Preparation of Ivacaftor Stock solution (120 µg/ml):

Take 12 mg of Ivacaftor in 100 ml volumetric flask and make the volume with diluent to produce 120 µg/ml.

Preparation of Ivacaftor Spiking Solutions:

From the above Ivacaftor 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 10 ml volumetric flask and make up the volume up to the mark with diluent to produce 0.6 µg/ml, 1.2 µg/ml, 1.8 µg/ml, 4.8 µg/ml, 12.0 µg/ml, 14.4 µg/ml, 19.20 µg/ml and 24.0 µg/ml. Calibration standards and quality control (QC) samples were prepared by spiking blank plasma with working stock dilutions of analytes to produce 60 ng/ml, 120 ng/ml, 180 ng/ml, 480 ng/ml, 1200 ng/ml, 1440 ng/ml, 1920 ng/ml and 2400 ng/ml.

Tezacaftor

Preparation of Tezacaftor Stock solution (600 µg/ml):

Take 60 mg of Tezacaftor in 100 ml volumetric flask and make the volume with diluent to produce 600µg/ml.

Preparation of Tezacaftor Spiking Solutions:

From the above Tezacaftor 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 10 ml volumetric flask and make up the volume up to the mark with diluent to produce 3µg/ml, 6 µg/ml, 9 µg/ml, 24 µg/ml, 60 µg/ml, 72 µg/ml, 96 µg/ml and 120 µg/ml.

Calibration standards and quality control (QC) samples were prepared by spiking blank plasma with working stock dilutions of analytes to produce 300ng/ml, 600 ng/ml, 900 ng/ml, 2400 ng/ml, 6000 ng/ml, 7200 ng/ml, 9600 ng/ml and 12000 ng/ml.

Preparation of internal standard Solution (Lumacaftor):

Stock-1: Take 50 mg of Lumacaftor 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.

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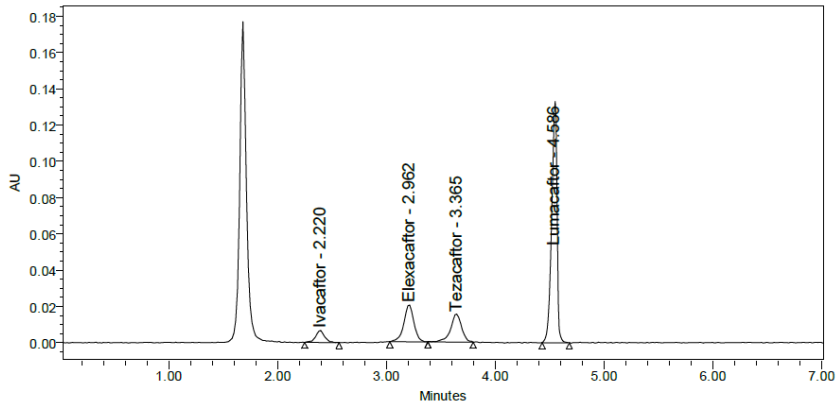
Final concentration:
From the above solution, take 0.5ml of solution and spiking blank plasma with working stock dilutions of analyte to produce 10µg/ml ISD concentration.

(OPTIMIZED METHOD):

Chromatographic conditions
Mobile phase : 0.1%Triethylamine pH (2.1): Acetonitrile (70:30)
Flow rate : 1.0ml/min
Column : kromasil C18 (250 x 4.7 mm, 5µ)
Detector wavelength : 272nm
Column temperature : 30^{0}C
Injection volume : 10µL



VALIDATION:
METHOD VALIDATION

System suitability of Elexacaftor, Tezacaftor and Ivacaftor

Sample Name	File Name	Analyte Area	Analyte RT (min)	ISTD Area	ISTD RT (min)	Area Ratio
MEAN			8.030 4.84 7.930		3.983 3.983 3.983	0.05057 0.04290 0.01417
SD			0.1146 0.0093 0.1144		0.0091 0.0091 0.0091	0.000315 0.000111 0.000102
%CV			1.44 0.19 1.43		0.23 0.23 0.23	0.62 0.26 0.72

Table no 1: System Suitability of Elexacaftor, Tezacaftor and Ivacaftor

Matrix factor evaluation of Elexacaftor, Tezacaftor and Ivacaftor

Acquisition Batch ID		Date	
S. No.	Plasma Lot No.	HQC	LQC
		Nominal Concentration (µg/mL)	
		12.921 8.900 1.920	1.304 0.900 0.180

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		(11.832-16.008) (8.160-11.040) (1.632-2.208)	(1.109-1.501) (0.765-1.035) (0.153-0.207)
		Calculated Concentration (µg/mL)	
n		18	18
Mean		13.9121 9.5932 1.9132	1.2957 0.8962 0.1799
SD		0.01131 0.45842 0.01818	0.00934 0.05067 0.01316
% CV		**0.08** **4.78** **0.95**	**0.72** **5.65** **7.31**
% Mean Accuracy		**99.94** **99.93** **99.65**	**99.29** **99.57** **99.97**
No. of QC Failed		**0**	**0**

Table no 2: Matrix factor evaluation of Elexacaftor, Tezacaftor and Ivacaftor

2. LINEARITY

Linearity of Elexacaftor, Tezacaftor and Ivacaftor

Batch ID	STD1	STD2	STD3	STD4	STD5	STD6	STD7	STD8
	Standard Concentration (ng/mL)							
	0.434 0.300 0.060	0.870 0.600 **0.120**	1.305 0.900 **0.180**	3.480 2.400 **0.480**	8.700 6.000 **1.200**	10.440 7.200 **1.440**	13.920 9.600 **1.920**	17.400 12.000 **2.400**
	Standard Concentration Range (ng/mL)							
	(0.348-0.522) (0.240-0.360) **(0.048-0.072)**	(0.740-1.001) (0.510-0.690) **(0.102-0.138)**	(1.109-1.501) (0.765-1.035) **(0.153-0.207)**	(2.98-4.00) (2.040-2.760) **(0.408-0.552)**	(7.395-10.005) (5.100-6.900) **(1.020-1.380)**	(8.874-12.006) (6.120-8.280) **(1.224-1.656)**	(11.832-16.008) (8.160-11.040) **(1.632-2.208)**	(14.790-20.010) (10.200-13.800) **(2.040-2.760)**
	Back Calculated Concentration (ng/mL)							
n	3	3	3	3	3	3	3	3
Mean	0.3013 0.4313 **0.0603**	0.6057 0.8720 **0.1193**	0.9007 1.3007 **0.1810**	2.4020 3.493 **0.4840**	5.9960 8.7037 **1.1983**	7.1363 10.4367 **1.4330**	9.5957 13.9127 **1.9103**	12.0847 17.3937 **2.3927**
SD	0.00833 0.00306 **0.00121**	0.01250 0.00300 **0.00379**	0.03495 0.00416 **0.00794**	0.02646 0.015 **0.03504**	0.12376 0.00473 **0.01012**	0.21996 0.02403 **0.00954**	0.02401 0.01656 **0.03620**	0.14917 0.01710 **0.02386**
%CV	**2.76** **0.71** **2.00**	**2.06** **0.34** **3.17**	**3.88** **0.32** **4.39**	**1.10** **0.44** **7.24**	**2.06** **0.05** **0.84**	**3.08** **0.23** **0.67**	**0.25** **0.12** **1.89**	**1.23** **0.10** **1.00**
% Mean Accuracy	**100.44** **99.16** **100.44**	**100.94** **100.23** **99.44**	**100.07** **99.67** **100.56**	**100.08** **100.3** **100.83**	**99.93** **100.04** **99.86**	**99.12** **99.97** **99.51**	**99.95** **99.95** **99.50**	**100.71** **99.96** **99.69**

Table no 3: Linearity of Elexacaftor, Tezacaftor and Ivacaftor

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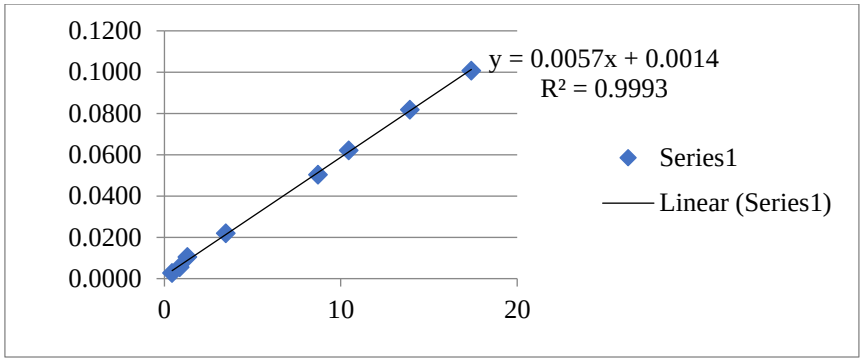

Fig no 1: calibration curve of Elexacaftor

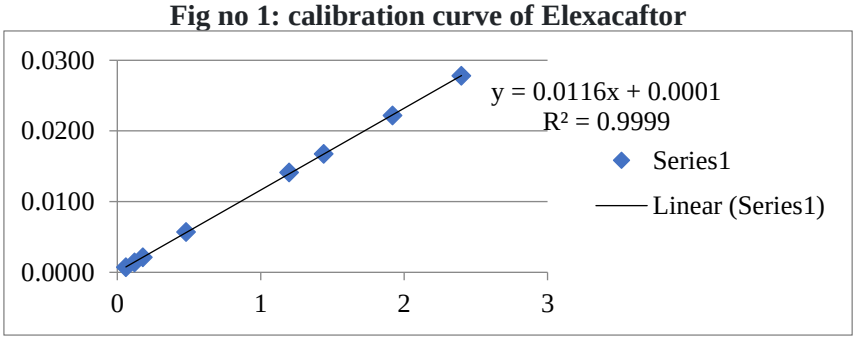

Fig no 2: calibration curve of Ivacaftor

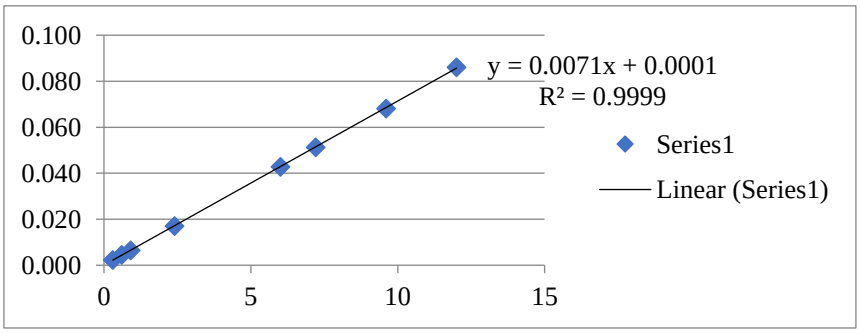

Fig No 3: calibration curve of Tezacaftor

3. Precision Accuracy (intra-day runs of Elexacaftor Tezacaftor Ivacaftor)

Acquisition Batch ID	Date	HQC	MQC1	LQC	LLOQ QC
		Nominal Concentration (µg/mL)			
		13.920 **9.600** **1.920**	**8.700** **6.000** **1.200**	**1.305** **0.900** **0.180**	**0.435** **0.300** **0.060**
		Nominal Concentration Range (µg/mL)			
		(11.83-216.008) (8.160-11.040) (1.632-2.208)	(7.395-10.00) (5.100-6.900) (1.020-1.380)	(1.109-1.501) (0.765-1.035) (0.153-0.207)	(0.348-0.522) (0.240-0.360) (0.048-0.072)
		Back Calculated Concentration (µg/mL)			
n		**6**	**6**	**6**	**6**
Mean		**13.9078** **9.5372** **1.9053**	**8.6947** **6.0543** **1.2013**	**1.3013** **0.8972** **0.1793**	**0.4390** **0.3015** **0.0598**
SD		**0.01158** **0.34739** **0.02007**	**0.00816** **0.13585** **0.00695**	**0.00836** **0.03419** **0.01294**	**0.01163** **0.02019** **0.00204**

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%CV	**0.08** **3.64** **1.05**	**0.09** **2.24** **0.58**	**0.64** **3.81** **7.22**	**2.65** **6.70** **3.41**
% Mean Accuracy	**99.91** **99.35** **99.24**	**99.94** **100.91** **100.11**	**99.72** **99.69** **99.63**	**100.92** **100.50** **99.72**
Between Batch Precision and Accuracy				
n	**18**	**18**	**18**	**18**
Mean	**13.9059** **9.5191** **1.9092**	**8.7001** **6.0046** **1.2061**	**1.2994** **0.8933** **0.1798**	**0.4359** **0.3009** **0.0596**
SD	**0.01388** **0.29819** **0.01850**	**0.01216** **0.18306** **0.01232**	**0.00961** **0.03337** **0.00882**	**0.00820** **0.01596** **0.00182**
%CV	**0.10** **3.13** **0.97**	**0.14** **3.05** **1.02**	**0.74** **3.74** **4.90**	**1.88** **5.30** **3.06**
% Mean Accuracy	**99.90** **99.16** **99.44**	**100.00** **100.08** **100.51**	**99.57** **99.26** **99.91**	**100.22** **100.30** **99.40**

Table No 4: Precision Data For Intra-Day Runs Of Elexacaftor, Tezacaftor And Ivacaftor

4. RECOVERY

Recovery of Elexacaftor, Tezacaftor and Ivacaftor

Acquisition Batch ID						
Replicate No.	**HQC**		**MQC1**		**LQC**	
	Un extracted Response	Extracted Response	Un extracted Response	Extracted Response	Un extracted Response	Extracted Response
n	6	6	6	6	6	6
Mean	26787 77276 93619	25524 76388 91674	16639 49424 58365	15757 47771 56774	2464 7276 13484	2344 7214 12683
SD	207.53 98.65 275.60	236.89 226.48 305.02	250.17 152.12 255.63	156.19 114.88 331.72	25.22 11.58 294.97	34.25 39.42 24.85
% CV	**0.77** **0.13** **0.29**	**0.93** **0.30** **0.33**	**1.50** **0.31** **0.44**	**0.99** **0.24** **0.58**	**1.02** **0.16** **2.19**	**1.46** **0.55** **0.20**
% Mean Recovery	95.29 98.85 97.92		94.70 96.66 97.27		95.10 99.14 94.06	
Overall % Mean Recovery	95.029 98.216 96.418					
Overall SD	0.3009 1.3581 2.0680					
Overall % CV	**0.32** **1.38** **2.14**					

Table No 5: Recovery of Elexacaftor, Tezacaftor And Ivacaftor

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Recovery - Internal standard

Acquisition Batch ID	**Date**	
S.No.	**Un extracted Area Ratio**	**Extracted Area Ratio**
n	6	6
Mean	1251680.2	1115060.5
SD	4568.50	2823.22
% CV	0.36	0.25
% Mean Recovery	89.09	

Table no 6: Recovery of Lumacaftor (IS)

5. STABILITIES

Long term stock solution stability at Zero Elexacaftor, Tezacaftor and Ivacaftor

Acquisition Batch ID	**Date**	
Replicate No.	**HQC**	**LQC**
	Nominal Concentration (µg/mL)	
	13.920 9.600 1.920	1.305 0.900 0.180
	Nominal Concentration Range (µg/mL)	
	(11.832-16.008) (8.160-11.040) (1.632-2.208)	(1.109-1.501) (0.765-1.035) (0.153-0.207)
	Calculated Concentration (µg/mL)	
n	6	6
Mean	13.909 9.6158 1.9038	1.3088 0.8948 0.1800
SD	0.00880 0.14799 0.02278	0.00662 0.06174 0.00888
% CV	**0.06** **1.54** **1.20**	**0.51** **6.90** **4.93**
% Mean Accuracy	**99.93** **100.16** **99.16**	**100.29** **99.43** **100.00**

Table no 7: Long term stock solution stability at Zero Elexacaftor, Tezacaftor and Ivacaftor

Matrix samples stability at -28±5 °C for 37 days Elexacaftor, Tezacaftor and Ivacaftor

Acquisition Batch ID			Date	
Replicate No.	**HQC**		**LQC**	
	Nominal Concentration (µg/mL)			
	13.921 9.600 1.920	13.920 9.600 1.920	1.305 0.900 0.180	1.305 0.900 0.180
	Nominal Concentration Range (µg/mL)			
	(11.832-16.007) (8.160-11.040) (1.632-2.208)	(11.832-16.008) (8.160-11.040) (1.632-2.208)	(1.109-1.501) (0.765-1.035) (0.153-0.207)	(1.109-1.501) (0.765-1.035) (0.153-0.207)
	Calculated Concentration (µg/mL)			
	Comparison Samples	Stability Samples	Comparison Samples	Stability Samples
n	6	6	6	6

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Mean	13.9185 9.6017 1.9014	13.8943 9.5483 1.9067	1.3062 0.9030 0.1787	1.2938 0.9032 0.1790
SD	0.01495 0.13060 0.03070	0.01412 0.34394 0.02125	0.00694 0.02884 0.00742	0.00733 0.06556 0.00912
% CV	**0.11** **1.36** **1.61**	**0.10** **3.60** **1.11**	**0.53** **3.19** **4.15**	**0.57** **7.26** **5.10**
%Mean Accuracy	**99.99** **100.02** **99.03**	**99.82** **99.46** **99.31**	**100.09** **100.33** **99.26**	**99.14** **100.35** **99.44**
% Mean Stability	**99.83** **99.44** **100.28**		**99.06** **100.02** **100.19**	

Table no 8: Matrix samples stability at -28±5 °C for 37 days Elexacaftor Tezacaftor Ivacaftor

Matrix samples stability at -80±5 °C for 37days (Elexacaftor Tezacaftor Ivacaftor)

Acquisition Batch ID			Date	
Replicate No.	**HQC**		**LQC**	
	Nominal Concentration (µg/mL)			
	13.920 9.600 1.920	13.920 9.600 1.920	1.305 0.900 0.180	1.305 0.900 0.180
	Nominal Concentration Range (µg/mL)			
	(11.832-16.008) (8.160-11.040) (1.632-2.208)	(11.832-16.008) (8.160-11.040) (1.632-2.208)	(1.109-1.501) (0.765-1.035) (0.153-0.207)	(1.109-1.501) (0.765-1.035) (0.153-0.207)
	Calculated Concentration (µg/mL)			
	Comparison Samples	Stability Samples	Comparison Samples	Stability Samples
n	6	6	6	6
Mean	13.9040 9.6022 1.9014	13.8938 9.5966 1.9067	1.3092 0.8980 0.1787	1.2973 0.9015 0.1790
SD	0.01420 0.34169 0.03070	0.01038 0.12330 0.02125	0.00747 0.04712 0.00742	0.00750 0.03001 0.00912
% CV	**0.10** **3.56** **1.61**	**0.07** **1.28** **1.11**	**0.57** **5.25** **4.15**	**0.58** **3.33** **5.10**
%Mean Accuracy	**99.89** **100.02** **99.03**	**99.81** **99.96** **99.31**	**100.32** **99.78** **99.26**	**99.41** **100.17** **99.44**
% Mean Stability	**99.93** **99.94** **100.28**		**99.10** **100.39** **100.19**	

Table no 9: Matrix samples stability at -80±5 °C for 37 days (Elexacaftor Tezacaftor Ivacaftor)

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SUMMARY AND CONCLUSION

Parameters	Elexacaftor	Ivacaftor	Tezacaftor	LIMIT
Linearity Range (ng/ml)	435-17400ng/ml	60-2400ng/ml	300-12000ng/ml	$R^2 < 1$
Regression coefficient	0.999	0.999	0.999	
Slope(m)	0.0057	0.061	0.122	
Intercept(c)	0.0014	0.001	0.001	
Regression equation (Y=mx+c)	y = 0.0057x + 0.0014	y = 0.0116x + 0.0001	y = 0.0071x + 0.0001	
Specificity	Specific	Specific	Specific	No interference of any peak
System precision %CV	0.08	1.05	3.64	NMT 15.0%
Method precision %CV	0.10	0.88	3.59	NMT 15.0%
Accuracy %recovery	96.41%	95.029%	98.21%	80-120%
LLOQ	435ng/ml	**60ng/ml**	**300ng/ml**	

Table no 10: summary for Elexacaftor, Tezacaftor and Ivacaftor

CONCLUSION

A straightforward and precise method was developed to quantify Elexacaftor, Ivacaftor, and Tezacaftor in human plasma using Lumacaftor as an internal standard. The retention times for Ivacaftor, Elexacaftor, and Tezacaftor were determined to be 2.391 minutes, 3.208 minutes, and 3.644 minutes, respectively. The %CV for Elexacaftor, Ivacaftor, and Tezacaftor were observed to be 0.08%, 1.05%, and 3.59%, respectively, while the %Recovery rates were measured at 96.41%, 95.029%, and 98.21%. The method exhibited excellent linearity over concentration ranges of 435-17400 ng/mL for Elexacaftor, 60-2400 ng/mL for Ivacaftor, and 300-1200 ng/mL for Tezacaftor (with an r2 value of 0.999). Lower limits of quantification were determined to be 435 ng/mL for Elexacaftor, 600 ng/mL for Ivacaftor, and 300 ng/mL for Tezacaftor, ensuring detection at levels commonly found in human plasma. Moreover, the method was validated according to ICH guidelines and demonstrated compliance within acceptable limits. In summary, this proposed method offers simplicity, speed, accuracy, precision, and suitability for pharmacokinetic and therapeutic drug monitoring in clinical laboratories.

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