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Simultaneous estimation of ivacaftor and tezacaftor in rat plasma by Liquid chromatography coupled with tandem-mass-spectrometry: Application to pharmacokinetic studies

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ABSTRACT

Background: Ivacaftor and Tezacaftor belong to the CFTR potentiator class, in combination approved to manage cystic fibrosis. **Objective:** To establish a sensitive LC-MS/MS approach for the synchronized analysis of Ivacaftor and Tezacaftor and its appliance to rat pharmacokinetic investigation. **Methodology:** Method is developed with protein precipitation by acetonitrile and Ivacaftor-d4, Tezacaftor-d4 are used as internal standards. Separation is done on an Eclipse plus C18 analysis column (100 mm × 4.6 mm 1.8 μm) with a mobile phase consisting of 0.1% trifluoroacetic acid: acetonitrile (ratio 60:40, v/v, and pH 2.5) and flow stream of 1.0 mL/min at ambient temperature. **Results:** The approach developed showed fine calibration curve in the quantity range of 1.5-22.53 ng/mL (r2 – 0.99974) for ivacaftor and 1-15.02 ng/mL (r2 – 0.99988) for tezacaftor and the accuracy and precision meets F.D.A guidelines. **Conclusion:** The newly designed and validated approach was simple, fast and applied effectively for rat pharmacokinetic investigation.

Keywords: Ivacaftor, Tezacaftor, LC-MS/MS, Method Validation, Pharmacokinetic study, Rat plasma

BACKGROUND

Cystic fibrosis, a progressive genetic disease, induces chronic lung infections and reduces breathing capacity over time.[1] Cystic fibrosis is caused by variations in the transmembrane conductance regulator (cystic fibrosis transmembrane regulator [C.F.T.R]) gene for cystic fibrosis.

C.F.T.R protein regulator works as an ion channel that controls the volume of liquid on the epithelial surfaces by inhibiting sodium absorption and chloride secretion,[2] resulting in thicker and stickier mucus than usual, which is difficult to remove from the lungs by cough leading to difficulty in breathing and severe lung infections.

Symdeko tablet formulation (labeled claim: 150 mg ivacaftor and 100 mg tezacaftor) was approved by Food and Drug Administration (FDA) in 2018.[3,4] Symdeko tablet is suggested for treating patients (aged 12 or older) with cystic fibrosis, patients with homozygous mutation (F508del), or mutation in the C.F.T.R gene. Ivacaftor [Figure 1] is a chloride channel agonist.[5-7] Tezacaftor [Figure 1] is a corrector of C.F.T.R protein,[8] cumulative effects of ivacaftor, and tezacaftor stimulates the C.F.T.R protein functions on the cell's surface, resulting in increased transport of chloride out of the body.[9-11]

To the best of our literature search, stability indicating RP-HPLC method,[12,13] UPLC[14] and UV spectrophotometric methods[15] were published to quantify tezacaftor and ivacaftor simultaneously in tablet formulations. For most of the analytes, liquid chromatography with mass spectrometry is considered as the most sensitive and specific approach compared to all other techniques. Liquid chromatography with mass spectrometry becomes the first option of quantitation for drugs in biological matrices. Three LC-M.S methods have been

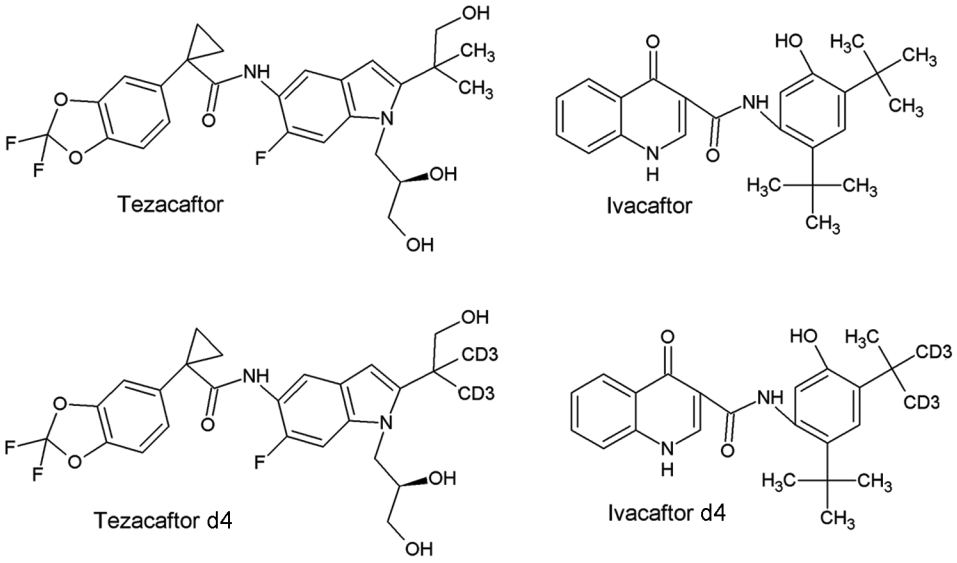


Figure 1: Structures of selected analytes and internal standards

published to quantify ivacaftor, major metabolites of ivacaftor and lumacaftor in cystic fibrosis patient's biological fluids.[16-18]

For the best of our literature, the pharmacokinetics of ivacaftor and tezacaftor has not been tested by liquid chromatography mass spectrometry (LC-MS). A simple, rapid and sensitive LC-MS-MS method for the simultaneous estimation of ivacaftor and tezacaftor in rat plasma have been developed and validated through this investigation.

MATERIALS AND METHODS

Drugs and Chemicals

The reference ivacaftor (99.99% purity) and tezacaftor (99.97% purity) standards were obtained from Cadila Healthcare Ltd., Ahmedabad, India. Ivacaftor-d4 (internal standard for ivacaftor, 99.98% purity) and tezacaftor-d4 (internal standard for tezacaftor, 99.97% purity) were obtained from Glenmark, Mumbai, India. HPLC grade acetonitrile (99.8%), phosphoric acid (99.7%) and Trifluoroacetic acid (99.5% purity) were got from Merck chemical division (Mumbai, India). Purified HPLC mark water was obtained by Milli-Q (Milli Q system, USA) water purification system.

Instrumentation

Waters, Alliance E2695 model HPLC (Waters Corporation, Milford, USA) provided with column oven, auto sampler and degasser was operated for analysis. The HPLC system was coupled to SCIEX QTRAP 5500 mass spectrometer (SCIEX, Canada) equipped with electrospray ionization interface. Analyst 1.6 software was used for the interpretation of chromatographic data. Eclipse plus C18 analysis column (100 × 4.6 mm and 1.8 µm dimension; Agilent Technologies, USA) was used for separation and analysis.

HPLC Conditions

Mobile phase was a mixture of 0.1% Trifluoroacetic acid and Acetonitrile (ratio 60:40 *v/v*), for which pH was set as 2.5. Acetonitrile and water combination was used as diluent at ratio of 70:30 volume by volume. Isocratic elution was done at 1.0 mL/min flow stream. Column and sample temperatures were kept at ambient temperature. The sample injection volume had been 50 µL.

Mass Spectrometer Conditions

The mass spectrometer was administered in the interface mode of positive ion electrospray ionization. Multiple reactions monitoring mode has been applied to quantify ivacaftor and tezacaftor. The ion transitions observed were m/z 393.46→360.29 for ivacaftor, m/z 521.29→420.55 for tezacaftor, m/z 397.68→360.17 for ivacaftor-d4 (internal standard for ivacaftor) and m/z 525.76→420.35 for tezacaftor- d4 (internal standard for tezacaftor). Working parameters have been set as Collision energy: 15 V for ivacaftor and ivacaftor-d4 and 14 V for tezacaftor and tezacaftor-d4, Ion spray voltage: 5500 V, Source temperature: 550°C, Drying gas temperature: 120–250°C, Collision gas: Nitrogen, Drying gas flow stream: 5 L/min, Declustering potential: 40 V, Entrance potential: 10V, Exit Potential: 7 V and Dwell time: 1 s.

Standard Solutions of Ivacaftor and Tezacaftor and Their Internal Standards

The stock standard solutions of ivacaftor (150 µg/mL), tezacaftor (100 µg/mL), ivacaftor-d4 (150 µg/mL), and tezacaftor-d4 (100 µg/mL) were prepared using diluent. Working standard solutions of the ivacaftor and tezacaftor mixture were made through serial dilution of the stock standard solution with diluent, with ivacaftor ranging from 1.50 to 22.53 ng/mL and tezacaftor ranging from 1.0 to 15.02 ng/mL. Working standard solutions of the ivacaftor-d4 (15.02 ng/mL) and tezacaftor-d4 (10.01 ng/mL) were made by diluting aptly stock standard solutions of ivacaftor-d4 and tezacaftor-d4 with diluent. All prepared standard solutions are stored at 4°C and bring to normal room temperature before using it.

Calibration Standard Solutions of Ivacaftor and Tezacaftor

Calibration standard solutions of ivacaftor (1.50, 3.76, 7.51, 11.27, 15.02, 18.78 and 22.53 ng/mL) and tezacaftor (1.0, 2.5, 5.01, 7.51, 10.01, 12.51 and 15.02 ng/mL) were made by spiking the rat plasma (100 µL) with correct volumes of mixed standard solution of ivacaftor and tezacaftor. To every calibration standard solution, appropriate volume of mixed internal standard solution with concentration of 15.02 ng/mL (ivacaftor-d4) and 10.01 ng/mL (tezacaftor-d4) was added. All the solutions were stored at –80°C and prior to analysis they are brought to ambient temperature.

Quality Control Samples of Ivacaftor and Tezacaftor

Samples of quality control were made as explained above in the similar way at concentrations corresponding to 1.50 (lower limits of quantitation [LLOQ]) ng/mL, 4.51 (lowest quality-control [LQC]) ng/mL, 12.02 (medium quality-control [MQC]) ng/mL and 18.53 (higher quality-control [HQC]) ng/mL for ivacaftor and 1.0 (LLOQ) ng/mL, 3.01 (LQC) ng/mL, 8.01 (MQC) ng/mL and 14.02 (HQC) ng/mL for tezacaftor. All the solutions were stock up at –80°C and prior to analysis they are brought to ambient temperature.

Preparation of Sample for Analysis

Added 100 µL of rat plasma, 0.5 mL of acetonitrile and 400 µL of internal standard and blended using vortex cyclomixer in a 1.5 mL centrifuge tube.

The blend was centrifuged at 4000 rpm for 15–20 min. The supernatant solution was collected in an HPLC vial, and the container was filled with 50 µL of prepared sample.

Validation of Method

Following the ICH[19] and FDA[20] bio-analytical method validation principles, the approach was validated to reveal the system appropriateness, auto sampler carryover, sensitivity, specificity, matrix consequence, linearity, precision, accuracy, extraction recovery, stability, and ruggedness.

Suitability of the system

System suitability has been made to ensure the LC-MS system is functioning well by producing accurate and precise results. For this, MQC sample was injected in five replicates. The percent coefficient of variation (CV) was computed for the retention time and area response of ivacaftor, tezacaftor and their internal standards (ivacaftor-d4 and tezacaftor-d4).

Auto sample carryover

The carryover of the auto sample was tested by injecting a blank rat plasma sample after HQC tests. The carryover of ivacaftor and tezacaftor should be lower than 20% mean peak area of ivacaftor and tezacaftor in LLOQ sample. The carryover of internal standards (ivacaftor-d4 and tezacaftor-d4) should be lower than 1% of mean peak area.

Screening of biological matrix (specificity)

This test was done to indicate that the blank endogenous plasma components were not chromatographically interacted with ivacaftor, tezacaftor and their internal standards. Interference from endogenous rat plasma components was assessed through evaluating 6 individual blank rat plasma samples and LLOQ sample.

Sensitivity

LLOQ was the limit to quantify the molecule accurately and precisely. Signal to noise ratio should be at least 10. The LLOQ value was evaluated by analyzing LLOQ level samples in six replicates.

Matrix effect

The matrix consequence of rat plasma on the simultaneous analysis of ivacaftor and tezacaftor was evaluated through comparison of peak areas of ivacaftor and tezacaftor in extracted blank plasma with that of obtained from ivacaftor and tezacaftor standard solutions. The matrix effect was studied at levels of LQC and HQC in 3 replicates.

Linearity

The linearity of ivacaftor and tezacaftor was evaluated in the series of 1.5 ng/mL - 22.53 ng/mL and 1 ng/mL - 15.02 ng/mL concentrations, respectively. Calibration curves of ivacaftor and tezacaftor were drawn by plotting peak area ratios (analyte peak area/internal standard peak area) against the different concentrations of analytes. The linearity was checked by linear regression analysis using $1/\times$ as weight.

Precision and accuracy of the method

The intra-day accuracy and precision were tested in six replicates in a single set using samples from HQC, MQC, LQC, and LLOQ concentration levels. The inter-day precision and accuracy were tested by analyzing HQC, MQC, LQC, and LLQC concentration samples on 3 successive separate batches. The precision was expressed by percent CV and the accuracy by percent recovery.

Recovery of internal standards and analytes

Ivacaftor recovery and tezacaftor recovery were evaluated by comparing peak areas of extracted LQC, MQC, and HQC samples with spiked LQC, MQC, and HQC samples after extraction. Ivacaftor-d4 and tezacaftor-d4 recovery were analyzed by equating peak areas of the pre-spiked samples to post-spiked samples.

Ruggedness

Ruggedness was conducted by repeating the analysis of HQC, MQC, LQC, and LLOQ samples in two different columns by two different analysts using the same bioanalytical procedure. The percent CV of recovery of ivacaftor and tezacaftor were determined.

Stability of ivacaftor and tezacaftor

The stability of ivacaftor and tezacaftor in rat plasma was evaluated by analyzing HQC, and LQC samples under different storage conditions to samples immediately after preparation (0 time stability), samples following 24 h of storage at room temperature (short period stability), samples after three freeze and thaw cycles, samples following 1 month of storage at –28°C (long period stability), samples following 1 month of storage at –80°C (long period stability).

Incurred sample reanalysis

As per the guidelines, the difference in the concentration between the initial value and the Incurred samples should be <±20% of their means for at least 67% of the repetitions. The samples were selected around the C_{max} and at elimination phase and a total of 18 samples were selected and reanalyzed as a separate batch. The change in the percentage can be calculated by the formula.

Pharmacokinetic study

The present study was applied to bioavailability test formulation. Nine Wistar male rats were randomly grouped into three Groups I, II, and III. Pharmacokinetic study was conducted in harmony with international animal care and maintenance standards. The rats were kept under controlled environment: temperature of 22 ± 2°C, 50 ± 10% relative humidity and 12 h dark and light cycle. All experimental procedures were performed in compliance with the Committee on Institutional Animal Ethics and carried out in accordance with the ethical guidelines set out in CPCSEA(Registration no.1074/PO/Re/S/05/CPCSEA), New Delhi, India.

Rats were administered orally with ivacaftor and tezacaftor at 150 mg and 100 mg per kg body weight, respectively. Blood samples (0.2 mL) from all rats were obtained in heparinized tubes at 0, 3, 6, 9, 12, 15, 18, 21, 24 h after dose, using dorsal pedal vein. To obtain the plasma sample, blood samples were centrifuged at 3000 rpm for 10 min. Prepared plasma samples were stored at −80°C. Within a month, the plasma samples were analyzed. The mean plasma concentration versus time curve after oral administration was shown in plasma concentration was shown in Figures 2 and 3 and Table 1. The pharmacokinetic parameters were measured using a noncompartmental statistical model results are shown in Table 1. The ensuing pharmacokinetic parameters determined are: C_{max} (maximum plasma concentration), T_{max} (time to reach peak concentration), $t_{1/2}$ (terminal elimination half-life), CL - plasma clearance, AUC_{0-t} - area below the curve from zero to time of last sampling, $AUC0_{-\infty}$ - *(*AUC_{0-t} extrapolated into infinity).

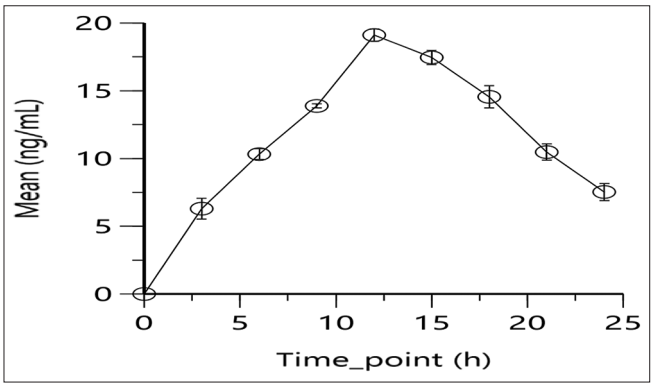


Figure 2: Concentration versus time profile of ivacaftor

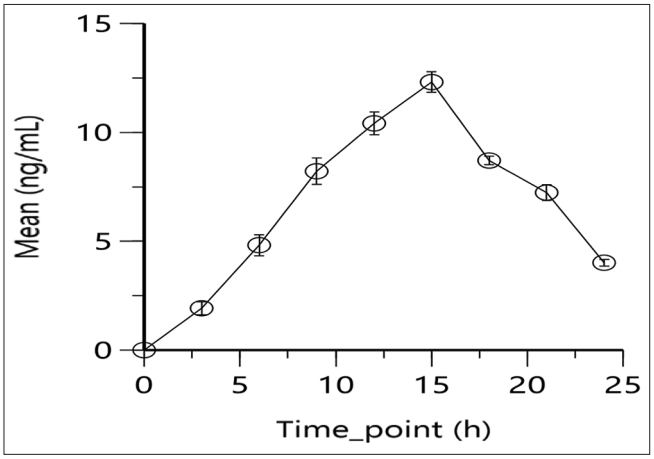


Figure 3: Concentration versus time profile of tezacaftor

RESULTS AND DISCUSSION

Method Establishing

The chromatography and mass spectrometer conditions were optimized to yield sensitive and efficient detection and quantification of ivacaftor and tezacaftor simultaneously. Deuterated (d4 substituted) ivacaftor-d4 and tezacaftor-d4 recovery was chosen as internal standards. These compounds are chemically equivalent to ivacaftor and tezacaftor [Figure 1]. They will prone to the same matrix effect as ivacaftor and tezacaftor. They will also chemically extract from the matrix in the same way to ivacaftor and tezacaftor. Therefore, the accuracy of the method will be enhanced and matrix effects are avoided.

To optimize electrospray ionization interface conditions for ivacaftor, tezacaftor and their respective internal standards (ivacaftor-d4 and tezacaftor-d4), quadrupole scan was done in positive and negative ion detection mode. Good response was attained in positive mode of ionization. The ion transitions observed for quantification were m/z 393.46→360.29 (ivacaftor), m/z 521.29→420.55 (tezacaftor), m/z 397.68→360.17 (ivacaftor-d4), and m/z 525.76→420.35 (tezacaftor-d4). Figure 4 shows the mass spectra of ivacaftor, tezacaftor, ivacaftor-d4, and tezacaftor-d4.

To achieve good peak shape and mass spectrometer response for ivacaftor and tezacaftor, trails were done with the following:

a. Two different columns (Luna C_{18} and Eclipse Plus C_{18})
b. Two different compositions of mobile phase (Acetonitrile with 0.1% Trifluoroacetic acid, pH 2.5 and Acetonitrile with 1.0% Formic acid, pH 3.5)
c. Ratios of acetonitrile (20% to 80%)
d. Injection volumes: 20 µl and 50 µl.

Finally, Eclipse plus C_{18} analysis column (100 × 4.6 mm, 1.8 µm dimensions) was chosen for separation and analysis. The mobile phase with 0.1% Trifluoroacetic acid: acetonitrile (ratio

Table 1: Pharmacokinetic parameters for ivacaftor and tezacaftor

Parameter	Ivacaftor	Tezacaftor
C_{max} (ng/mL)	19.11±0.77	12.31±0.59
t_{max} (h)	12.04±0.23	15.12±0.20
AUC_{0-t}(ng/mL/h)	287.57±1.45	166.88±1.64
$AUC_{0-\infty}$(ng/mL/h)	356.16±1.13	197.97±1.38
T 1/2(h)	6.31±0.53	5.36±0.27
Cl/F(mL/h/kg)	421154.68±2.71	505121.03±1.28

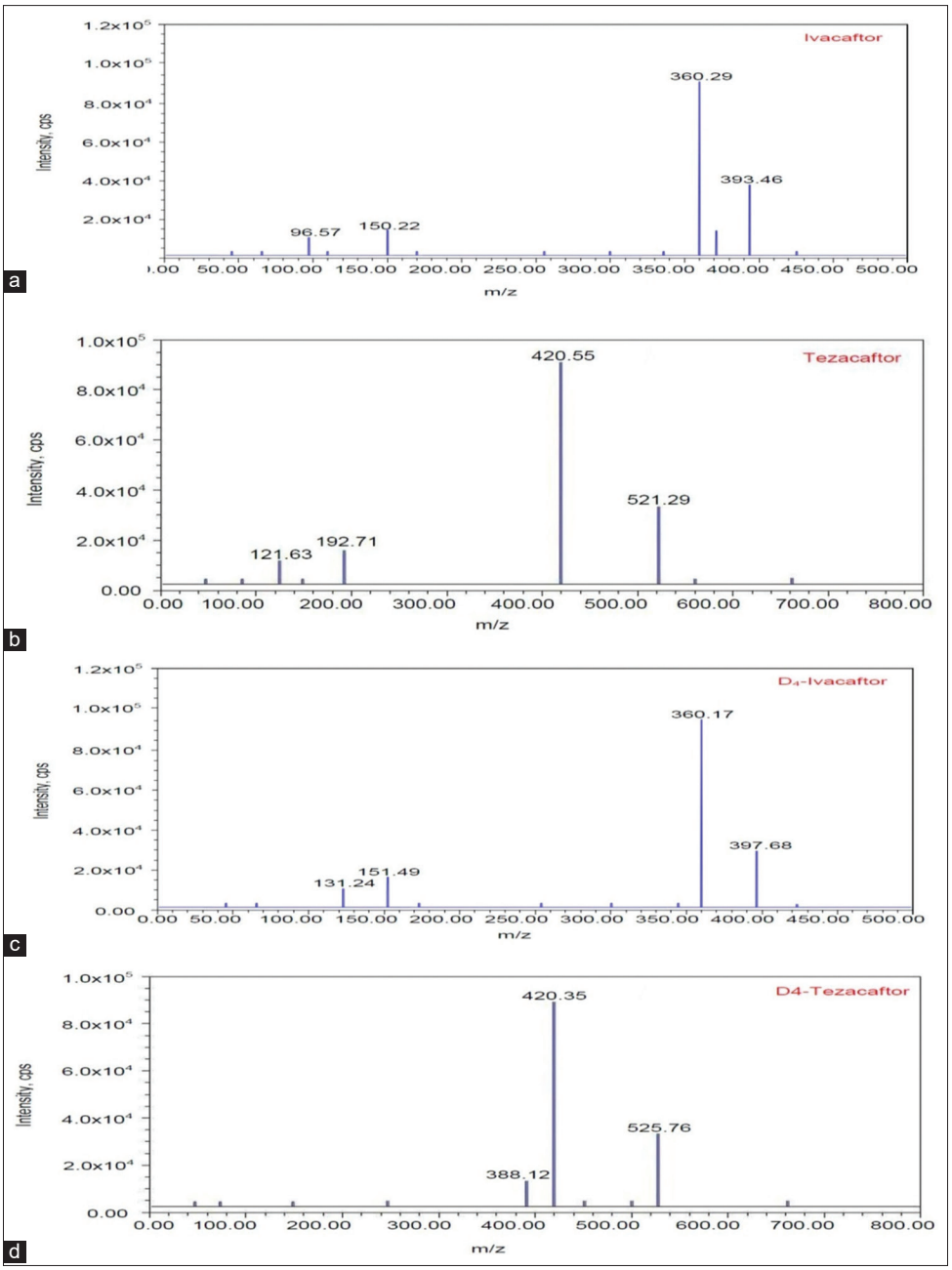


Figure 4: Mass spectra of (a) Ivacaftor (b) Tezacaftor (c) d-4 Ivacaftor (d) d-4 Tezacaftor

60:40, *v/v*) was selected and directed at an isocratic flow stream of 1.0 mL/min with 50 µL of sample injection volume. The retention times of ivacaftor, ivacaftor-d4, tezacaftor and tezacaftor-d4 were 5.128 min, 5.156 min, 6.028 min and 6.084 min, correspondingly, with 8 min run time, presented in [Figure 5].

Validation of Method

System suitability

The percentage CV values were less than 2.0% for retention times of ivacaftor, tezacaftor and their internal standards (ivacaftor-d4 and tezacaftor-d4). Furthermore, the percentage CV for area ratio was less than 2% [Table 2]. Hence, the system passed the system suitability test.

Auto sample carryover

Peak area response of ivacaftor, tezacaftor, ivacaftor-d4 and tezacaftor-d4 was not observed in the blank rat plasma sample after successive injections of HQC at the retention times of ivacaftor, tezacaftor, ivacaftor-d4 and tezacaftor-d4. Therefore, this method does not exhibit auto sample carryover.

Specificity

Interfering peaks were not observed at ivacaftor, tezacaftor, ivacaftor-d4 and tezacaftor-d4 retention times in the chromatogram of blank rat plasma [Figure 6]. Thus, proved specificity of the method to analyze ivacaftor and tezacaftor simultaneously.

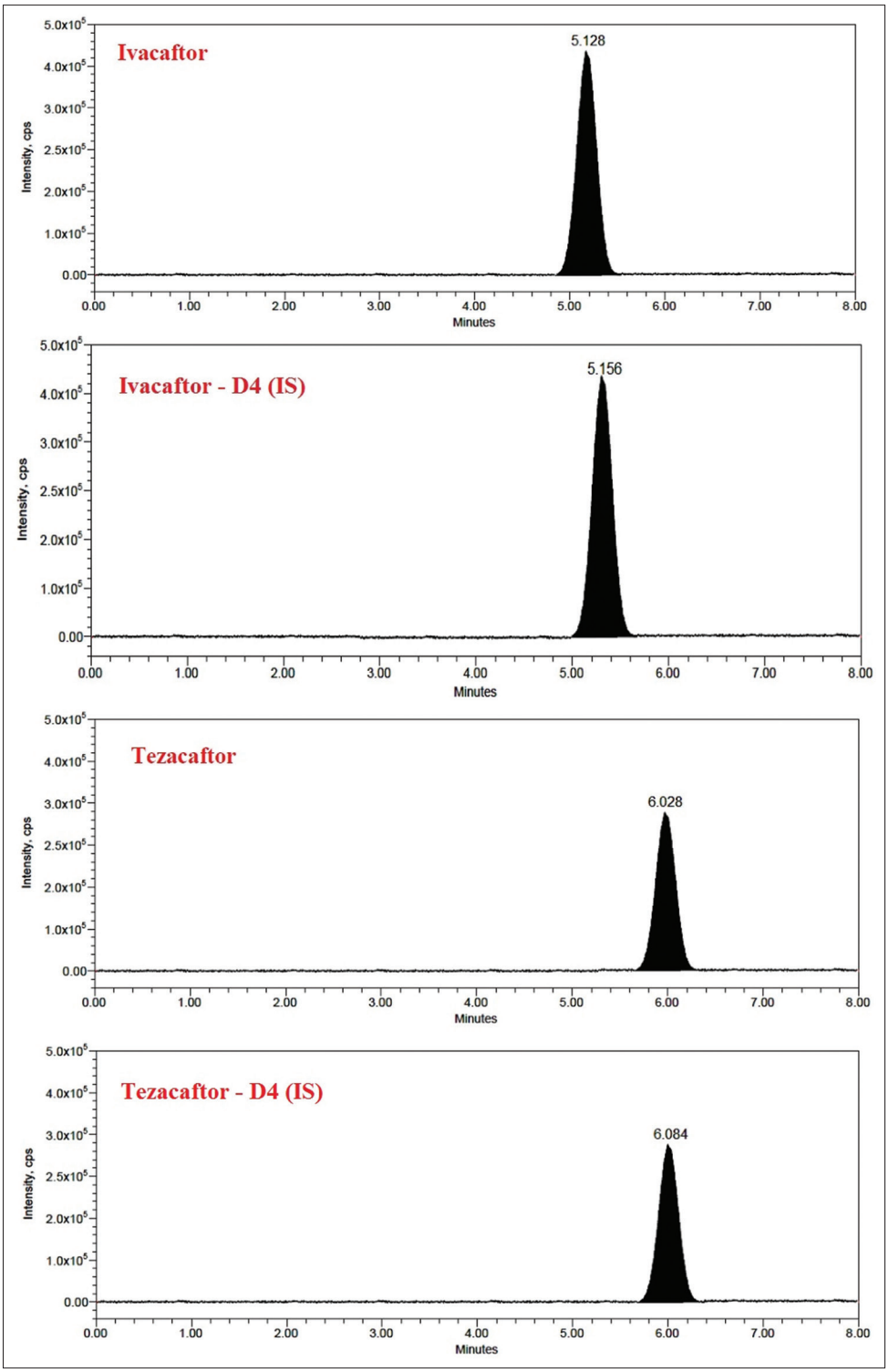


Figure 5: Chromatogram obtained with optimized conditions

Table 2: Outcomes of system suitability test

Statistical value	Retention time of		Area ratio*	Retention time of		Area ratio*
	Ivacaftor	Ivacaftor-d4		Tezacaftor	Tezacaftor-d4	
Mean**	5.165	5.163	0.9959	6.0845	6.0965	0.9886
SD	0.00251	0.00836	0.01089	0.00432	0.00226	0.00317
%CV	0.049	0.162	1.094	0.071	0.037	0.321

*Analyte peak area/internal standard peak area; **mean of five determinations

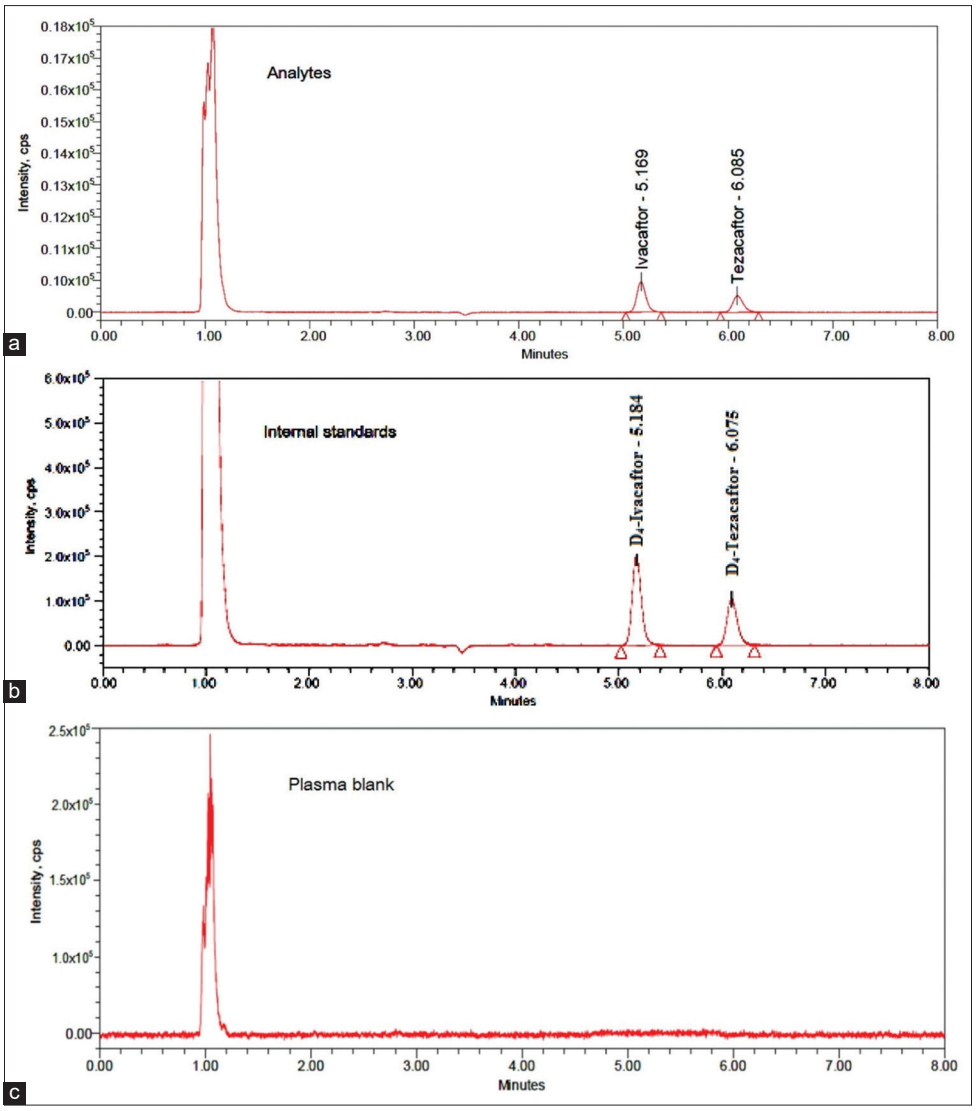


Figure 6: Chromatogram of (a) analytes (b) internal standard (c) plasma blank

Table 3: Outcomes of sensitivity test

Parameter	Ivacaftor	Tezacaftor
LLOQ value* (ng/mL)	1.5	1.0
Accuracy* (% Recovery)	99.58	99.45
Precision (% CV)	3.444	4.229

*Mean of six determinations

Sensitivity

The LLOQ values for ivacaftor and tezacaftor were 1.5 ng/mL and 1.0 ng/mL, correspondingly.

The accuracy (% mean recovery) and precision (%CV) determined at LLOQ quantity level are revealed in Table 3. They are within the approved limits.

Effect of matrix

The mean recoveries of ivacaftor at LQC and HQC levels were 99.89% and 99.85% with a CV of 0.24% and 1.59%, respectively. The mean recovery of tezacaftor at the LQC and HQC levels were 99.92% and 99.95% with a CV of 0.13% and 0.35%, respectively. The percent mean recoveries and percent

Table 4: Outcomes of precision and accuracy test

Sample	Intra-day		Inter-day	
	Percent recovery*	Percent CV	Percent recovery**	Percent CV
Ivacaftor				
LLOQ	99.78	5.43	99.64	5.36
LQC	99.89	0.89	99.93	1.24
MQC	100.06	0.35	100.32	0.54
HQC	99.95	0.18	100.25	1.02
Tezacaftor				
LLOQ	99.85	0.62	99.58	0.66
LQC	99.95	0.37	99.93	1.24
MQC	100.32	0.29	100.08	0.67
HQC	99.98	0.08	100.25	0.24

*Mean of six determined values; **Mean of 18 determined values

Table 5: Recovery of ivacaftor and tezacaftor

Statistical values	HQC sample		MQC sample		LQC sample	
	Pre-spiked	Post-spiked	Pre-spiked	Post-spiked	Pre-spiked	Post-spiked
ivacaftor						
Average response*	3.017×10^5	3.133×10^5	2.028×10^5	2.125×10^5	1.052×10^5	1.048×10^5
% Recovery*	96.29		95.43		100.38	
Mean recovery			97.36			
%CV			1.13			
Tezacaftor						
Average response*	2.255×10^5	2.353×10^5	1.503×10^5	1.622×10^5	0.753×10^5	0.755×10^5
% Recovery*	95.83		92.66		99.73	
Mean recovery			96.07			
% CV			0.11			

*Mean of six determined values

CV values indicated that there was no significant effect of the matrix on the bioanalytical methodology for simultaneous evaluation of ivacaftor and tezacaftor.

Linearity

The ivacaftor and tezacaftor calibration curves were linear through the concentration series of 1.5–22.53 ng/mL and 1–15.02 ng/mL, respectively. Equations of ivacaftor and tezacaftor calibration curves were:

$$\text{Peak area ratio} = 0.066899\,x + 0.00095;\ r^2 \text{ (correlation coefficient)} = 0.99988 - \text{ivacaftor}$$

$$\text{Peak area ratio} = 0.098654\,x + 0.00228;\ r^2 \text{ (correlation coefficient)} = 0.99974 - \text{tezacaftor}$$

Where x = concentration of ivacaftor or tezacaftor in plasma (ng/mL)

The correlation coefficient values demonstrated better linearity of ivacaftor and tezacaftor in the studied concentration range.

Precision and accuracy

The intra- and inter-day accuracy was ranged through 99.64% and 100.32% for ivacaftor, and 99.85% and 100.32% for tezacaftor [Table 4]. The intra- and inter-day precision was ranged through 0.18% and 5.43% for ivacaftor, and 0.08% and 1.24% for tezacaftor [Table 4]. The accuracy and precision results met the acceptable bioanalytical criteria.

Recovery of internal standards and analytes

The recoveries for ivacaftor (95.43–100.38%) and tezacaftor (92.66–99.73%) at LOQ, MOQ and HOQ levels and mean recovery of ivacaftor (97.36%) and tezacaftor (96.07%) are summarized in Table 5. The percent CV was 1.13% for ivacaftor and 0.11% for tezacaftor**.** The extraction recoveries and percent CV for ivacaftor-d4 and tezacaftor-d4 are shown in Table 6. The results demonstrated that the bioanalytical method had good extraction efficiency. This also showed that the recovery was not dependent on concentration.

Ruggedness

The percent recoveries and percent CV of ivacaftor and tezacaftor determined with two different analysts and on two different columns were within acceptable criteria in HQC, MQC, LQC and LLOQ samples [Table 7]. The percent recoveries ranged from 99.21% to 100.26% for ivacaftor and 99.54% to 100.29% for tezacaftor. The percent CV values ranged from 0.19% to 1.96% for ivacaftor and 0.15% to 0.46% for tezacaftor. The results proved method's ruggedness.

Table 6: Recovery of ivacaftor-d4 and tezacaftor-d4

Statistical values	Response of pre-spiked	Response of post-spiked
Ivacaftor-d4		
Average response*	2.038×10^5	2.148×10^5
% Recovery*	94.87	
% CV	0.65	
Tezacaftor-d4		
Average response*	1.504×10^5	1.540×10^5
% Recovery*	97.66	
% CV	1.09	

*Mean of six determined values

Table 7: Outcomes of ruggedness test

Sample	Different columns		Different analysts	
	Percent recovery*	Percent CV	Percent recovery*	Percent CV
Ivacaftor				
LLOQ	100.21	1.58	99.21	1.96
LQC	100.14	1.44	99.37	0.74
MQC	100.26	0.34	99.58	0.32
HQC	100.01	0.32	99.85	0.19
Tezacaftor				
LLOQ	100.16	0.41	99.87	0.46
LQC	100.08	0.40	99.54	0.34
MQC	100.29	0.15	99.68	0.21
HQC	100.13	0.15	99.95	0.18

*Mean of six determined values

Table 8: Outcomes of stability tests

Stability	Mean peak area of stability sample	%CV	Mean peak area at zero time	%CV	% Deviation
Ivacaftor					
Auto sampler stability					
LQC	1.041	1.26	1.024	1.16	1.66
MQC	2.042	1.68	2.018	1.02	1.18
HQC	3.036	1.26	3.025	1.20	1.1
Short term stability					
LQC	0.989	0.26	0.986	0.28	0.30
HQC	2.989	0.24	2.985	0.21	0.13
Freeze thaw stability					
LQC	1.013	1.23	1.005	1.28	0.79
HQC	3.011	0.10	3.014	0.18	-0.09
LT at -20					
LQC	0.766	1.46	0.752	1.21	1.86
HQC	2.786	0.18	2.781	0.15	0.18
LT at -80					
LQC	0.687	1.23	0.699	1.28	-0.43
HQC	2.682	0.40	2.679	0.49	0.11
Tezacaftor					
Auto sampler stability					
LQC	0.753	1.52	0.751	1.28	0.26
MQC	1.507	1.75	1.504	1.65	0.19
HQC	2.255	1.57	2.251	1.45	0.17
Short term stability					
LQC	0.710	0.65	0.712	0.68	-0.28
HQC	2.213	0.29	2.205	0.61	0.36
Freeze Thaw stability					
LQC	0.754	1.35	0.751	1.18	0.39
HQC	2.255	0.98	2.256	0.62	-0.04
LT at -20					
LQC	0.492	0.79	0.491	0.44	0.20
HQC	1.939	0.54	1.932	1.21	0.36
LT at -80					
LQC	0.389	1.59	0.384	1.64	1.30
HQC	1.866	0.87	1.868	1.14	–0.10

*Mean of six determined values; **Mean of 24 determined values

Stability of ivacaftor and tezacaftor

The results of ivacaftor and tezacaftor stability tests were summarized in Table 8. The findings showed that ivacaftor and tezacaftor are durable in rat plasma under various storage conditions studied, including stored at room normal temperature for 24 h, and after freeze thaw, and long term storage for –80°C for 30 days.

Pharmacokinetic studies

The established and validated bioanalytical approach was productively applied to study the pharmacokinetics following oral administration of 150 mg (ivacaftor) and 100 mg (tezacaftor) per kg body weight to nine rats. The average plasma quantity versus time profile subsequent to oral dose administration of ivacaftor and tezacaftor are depicted in Figures 2 and 3. From the curve, it was found that ivacaftor and tezacaftor were detected up to 24 h after dosing. All the plasma concentrations of analytes were within the standard curve region and retained above LOQ for the entire sampling period. The pharmacokinetic parameters are given in Table 1. The method was adequate during the 24 h sampling period to determine the plasma concentration profiles of ivacaftor and tezacaftor.

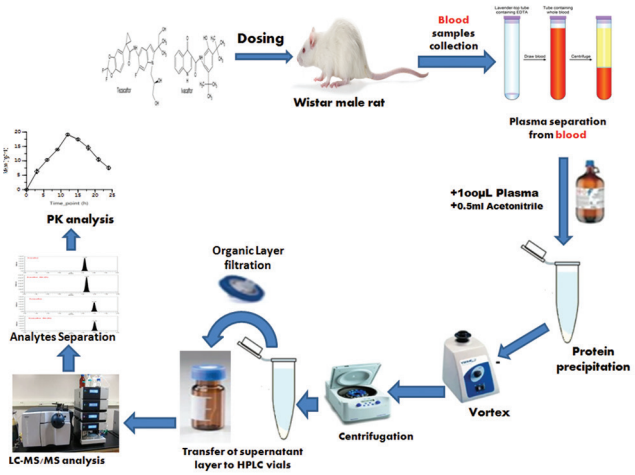
CONCLUSION

In this investigation, a simple and sensitive LC-MS method was established and validated to simultaneous quantify ivacaftor and tezacaftor in the sample plasma of rats. The method involved simple single step precipitation method using acetonitrile for sample preparation. The results showed satisfactory recovery as well as a lack of major matrix effects. The validation results are well within the criteria of acceptance. This method was extended effectively to the ivacaftor and tezacaftor pharmacokinetic study in rats.

SUMMARY

The present research is a novel LC-MS/MS system developed and approved by U.S. FDA for the determination of ivacaftor and tezacaftor in the plasma sample of rats. The drug is isolated from the endogenous components by an effortless protein precipitation step, and the drugs are tested for Eclipse plus C18 analysis column (100 × 4.6 mm and 1.8 µm thickness) with a mobile phase consisting of 0.1% of trifluoroacetic acid: acetonitrile (ratio 60:40, v/v, pH: 2.5) and pump at an isocratic rate of 1.0 mL/min of flow with a sample size of 50 µL. Validation parameters are within acceptable limits and show excellent linearity between 1.5 ng/mL and 22.53 ng/mL and 1 ng/mL to 15.02 ng/mL for ivacaftor and tezacaftor, respectively. The tmax values for ivacaftor and tezacaftor are 12.04±0.23 and 15.12±0.20 h and can be used to derive further dosing patterns and regimens. The method developed is quick, easy and rapid, and can be used routinely for bioanalytical research.

Pictorial Representation



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