RESEARCH ARTICLE

Stability Indicating LC-MS/MS Method Development and Validation for the Quantification of Cabotegravir in Biological Samples

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ABSTRACT

The major goal of current research study was to create a sensitive tandem mass spectrometric method using electrospray ionisation and liquid chromatography for quantifying cabotegravir in biological matrices. A stationary Phenomenex C18 column with dimensions of 50 × 4.6 mm and 5.0 µm particle size of was used to achieve chromatographic elution. With the flowing rate of 0.80 mL/min, isocratic separation was done using methanol and 0.10% V/V HCOOH in a fraction of 85:15 V/V as the mobile phasic system. For drug and internal standard separation, liquid-liquid extraction was carried out using methanol and ethyl acetate (1:4) solvent solution. On repeated reaction monitoring, fragment and product ionic values were seen at m/z 406.12→142.04 for cabotegravir and 450.12→160.03 for bictegravir internal standard. Drug's linearity graph had a r^2 value of 0.9998 and was rectilinear at concentrations between 400 and 16000 ng/mL. The inter- and intra-batch accuracy %relative standard deviation values ranged from 2.54 to 5.21. The percent recovery results of the lower quality control (LQC), median quality control (MQC), and higher quality control (HQC) sample solutions were 102.85, 97.84, and 94.27%, respectively. This approach has excellent recoveries. Studies on stability were processed under various circumstances, and stability values ranged from 92.93 to 103.89%. When exposed to various stability conditions, cabotegravir is more steady for a longer time, and the approach was successfully applicable to routine examination of cabotegravir in biological samples.

Keywords: HIV, Cabotegravir**,** LC/MS/MS, USFDA guideline, Stability, Linearity.

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INTRODUCTION

Rilpivirine and the cabotegravir, non-nucleoside reverse transcriptase inhibitor, also known as GSK1265744, are administered together as an HIV-1 integrase inhibitor. According to early cabotegravir study, dolutegravir had a higher oral bioavailability.[1,2] Later, cabotegravir was converted as a monthly intramuscular injection with a lengthy half-life. Inhibiting strand transfer of the viral genome into the host genome and stopping virus replication, cabotegravir interacts to the active site of HIV integrase. For the short-term therapy of HIV-1 in virologically suppressed people without a history of treatment failure or who have missed an injection of cabotegravir, oral cabotegravir and rilpivirine are recommended.[3,4] Rilpivirine and intramuscular extended-release cabotegravir are recommended as a full regimen for people with virological suppression who have never had therapy failure. Their present antiretroviral medication is supposed to be replaced with the intramuscular form. Chemically cabotegravir designated as {[(2*R*, 3*S*)- 1-[*N*- (2-methylpropyl) (4-aminobenzene) sulfonamido] -3-({ [(3*S*) -oxolan-3-yloxy] carbonyl}amino)-4-phenylbutan-2-yl]oxy} phosphonic acid with molecular weight and formula of 405.358 g·mol^{-1} and $C_{19}H_{17}F_2N_3O_5$ (Figure 1). The T_{max} of cabotegravir taken orally is 3 hours, the C_{max} is 8.0 g/mL, and the AUC is 145 g*h/mL. Cabotegravir for intramuscular extended release has a T_{max} of 7 days, a C_{max} of 8.0 g/ml, and an AUC of 1591 g.h/ml. UGT1A1 performs 67% of the glucuronidation, while UGT1A9 performs 33%, converting cabotegravir to the M1 and M2 metabolites.[5-7]

Literature on cabotegravir reveals that, one quality by design method was developed for the determination of its related substance and degradation products,[8] high-performance liquid chromatography (HPLC) method was reported for the determination it in combination with rilpivirine[9] and a multiplex UHPLC-MS/MS assay for the monitoring of the plasma concentrations of the antiretroviral drugs bictegravir, cabotegravir, doravirine, and rilpivirine in people living with HIV.[10] In this connection development of specific method like LC-MS/MS is essential for quantifying cabotegravir in biological matrices.

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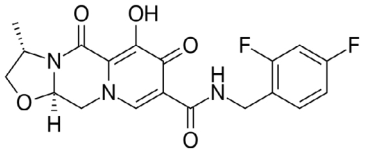

Figure 1: Chemical structure of cabotegravir

MATERIALS AND METHOD

Reagent Chemicals

Dr.laboratory Reddy's in Hyderabad, India provided the cabotegravir (purity: 99.87%). From MSN Labs in Hyderabad, India, bictegravir (internal standard) of 99.94% was acquired. Formic acid of analytical quality and acetonitrile of HPLC grade were purchased from JT Bakers in Hyderabad, India. The Milli-Q®RO system's built-in water supply was used for the processing of the moveable phase and washing solvents.

LC-MS/MS Instrument

The current study used an LC-MS/MS SCIEX API4000 apparatus. It was furnished with a positive Electrospray ionisation source (ESI) and LC Shimadzu prominence, which includes, binary pumping system, oven system for column and a SIL HTIC autosampler. The software of analyst version 1.06.30 was combined to perform the quantification of the analysis, the collection of the data, and its integration.

Liquid Chromatographic System

Chromatographic isolation of components was accomplished on a C_{18} Phenomenex stationary phase having 50 × 4.60 mm dimensions and 5.0 µm particle size. Infusion volume was set to 5.0 µL. Flow rate and stationary phase oven temperatures were retained at 0.80 mL/min and 25.0°C. Isocratic separation was executed with methanol and 0.10% V/V HCOOH in a fraction of 85:15 V/V as mobile phasic system. Cabotegravir and bictegravir IS were eluted in a total run timing of 3 minutes. The autosampler and analytical stationary columns temperature conditions were monitored at 5.0 and 25.0°C correspondingly.

Mass Instrument Parameters

Both Cabotegravir and an internal standard were run through the mass spectrometer in +ve ionisation mode using the multiple reaction monitoring mode. The sheath gas and drying gas temperature conditions were both at 450°C in the altered mass instrument factors for cabotegravir and bictegravir. A 25.0 psi nebulizer pressure gauge was used. The drying and sheath gas flow ratings were 15.0 and 4.0 L/min, respectively. A 3.0 kV capillary voltage was used. Each transition has a 200 ms dwell period. For cabotegravir and IS, the collisional energy and fragmentor voltages were respectively 15 eV/15.0eV and 115.0 V/110 V. The precursor to product transitions were monitored at m/z 406.12→142.04 for cabotegravir and 450.12→160.03 for IS.

Linearity Standard Samples

A new stock sample of cabotegravir at 1.0 mg/mL was generated by making dissolve 100mg of medication in a 100 mL mobile phasic solvent. Linearity standard smples were created by spiking blank plasma with cabotegravir standard solution to obtain 400, 600, 1800, 3600, 6000, 9000, 12500, and 16000 ng/mL concentrations.

Quality Control Standards

These control standards were produced at 3 distinct quality control levels: LQC, MQC, and HQC. These quality control (QC) samples were generated according to calibration standards, corresponding to 8000, 1120 and 12000 ng/mL concentrations for MQC, LQC and HQC. The processed samples were kept at -200°C until the analysis time.

Method of Sample Preparation

In 200.0 µL sample of plasma and 100 µL of IS (1-µg/mL) were combined to create the sample solution, which was vortexed for two minutes. With methanol and ethyl acetate (1:4) as the solvent system, cabotegravir and IS were extracted. The solution was then centrifuged at 5000.0 rpm for 25 minutes. Following centrifugation, the organic layer was isolated and subjected for drying with a lyophilizer. The finished compound was put into pre-labeled vials after being made to soluble in 250.0 µL of mobile phasic system. Infusion of the vials into the LC-MS/MS apparatus was done while they were in an auto-sampler.

Method Validation

The characteristics selectivity, stability, specificity, linearity, matrix effect, precision, recovery, and accuracy were used to verify the devised technique.[11,12]

RESULTS AND DISCUSSION

Optimization of Mass Equipment Conditions

To get a greater sensitivity and better specificity for the determination of mass, we used the MRM in +ve mode of ionasation with cabotegravir. The solution of standard were infused into mass equipment using the pump of syringe to determine the precursor ions and product ions as ESI was most likely the greatest basis of ionisation for LC/MS/MS. The mass spectra of cabotegravir and IS's product ions, which were chosen as the detection ions, were obtained at m/z 142.05 and 160.03. In the meanwhile, to achieve a larger mass response, the constraints of the mass spectral settings (such as ion-spraying voltage, temperature, capillary voltage and heater gases, collision gas, curtain gas, and nebulizer gas) were tuned.

Selection of Internal Standard

Bictegravir was chosen as the IS in the current study because, as per the method's validation findings, no obvious intrusions were discovered at retaining drug and bictegravir time points. It also had parallel chromatographic behavior, ionisation, extraction effectiveness, and retaining action as cabotegravir.

Method Validation

Specificity

To gauge the specificity, samples of blank plasma from 6 separate human plasma groups were processed for spiking at

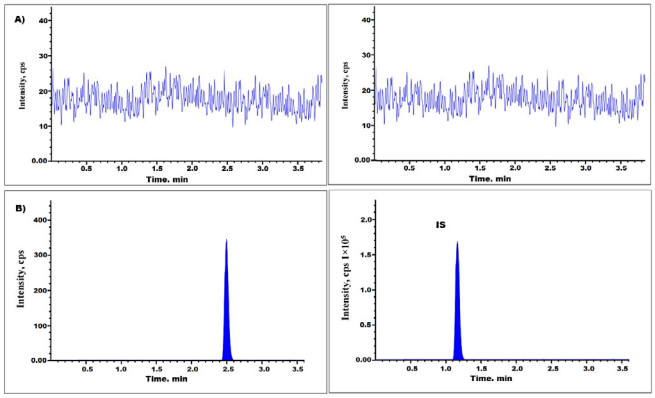

Figure 2: (A) Plasma blank and (B) LLoQ sample chromatogram

LLoQ (cabotegravir) and with IS. The retention periods of IS and cabotegravir were discovered to be 1.18 and 2.5 minutes, respectively, as shown in Figure 2. Analytical results for cabotegravir showed no discernible matrix material or internal standard incursion, and the responses of all intrusive peaks were lesser than 20.0% of LLoQQC samples.[13]

Linearity and sensitivity

The method's linearity for cabotegravir was developed, and it showed good linearity in a concentration array of 400 to 16000 ng/mL. The peak area ratios of cabotegravir to the IS vs concentration (x) with a $1/C^2$ weighting factors were used to create the linearity plots (Table 1). The calibration graphs' created technique linearity equation was y = 0.000099x + 0.00335 (Figure 3), with a r^2 finding of 0.9998. The LLoQ standard of cabotegravir was 400 ng/mL (signal to noises fraction > 10) and was sufficient for accurate quantification of cabotegravir in the analysis of plasma samples.[14-16]

Accuracy and precision

Six plasma samples spiked with cabotegravir at HoQ, MQC, LQC, and LLQC levels were tested in one batch and three subsequent batches to determine the precision and accuracy of intrabatch and interbatch measurements. The results of a precision and accuracy tests for cabotegravir quantitation are shown in Table 2. The inter- and intra-batch accuracy % relative standard deviation values ranged from 2.54 to 5.21.[17,18]

Extraction recovery

Prior to analysis, the biological samples were successfully pretreated. The extraction recoveries were evaluated by measuring the peak response fraction of HQC, MQC, and LQC level samples (n=06) of cabotegravir to an extracted spiking solutions at corresponding concentration levels. Peak response fraction of quality control sample solutions of plasma (n=06) to human plasma spiked sample solutions at different levels of concentration was used to determine the extraction recovery of IS in the same way. At high, medium, and low QC levels, the mean extraction recovery of cabotegravir was 94.27, 97.84, and 102.85%, respectively. At the concentration of 650 ng/mL, the average extraction recoveries of IS was 98.16%. The results were shown in Figures 4 to 6 and Table 3.[17]

Table 1: Linearity standard solutions for cabotegravir

LS-ID	*Concentration (ng/mL)*	*Average response*	*IS response*	*Area response*
LS -1	400	4832	115942	0.041676
LS -2	600	7202	115512	0.062349
LS -3	1800	21462	115643	0.185588
LS -4	3600	42459	115185	0.368616
LS -5	6000	68980	115943	0.594948
LS -6	9000	101631	115825	0.877453
LS -7	12500	144774	115741	1.250845
LS -8	16000	182846	115369	1.58488

LS: Linearity standard.

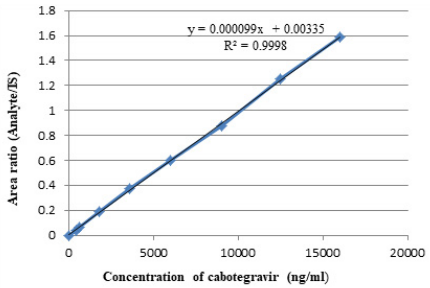

Figure 3: Linearity of cabotegravir

Matrix effect

Due to the method's accuracy, co-eluting matrix constituents may diminish or increase the ionisation procedure in the mass instrument, which may not result in a noticeable response in the blank matrix. As a result, the bictegravir normalised matrix factor was estimated in 08 distinct sources of human plasma, including two hemolytic batches and two lipemic. Results were displayed in Table 4 with the mean bictegravir normalised matrix factor for all analyte components present in the range of 1.05 to 0.96 with a percent[18] RSD of 4.91.

Stability

Both matrix-based samples and aqueous-based preparations were used to assess cabotegravir's stability. Stock solutions processed with diluent were unaffected at 1.0 to 10°C for

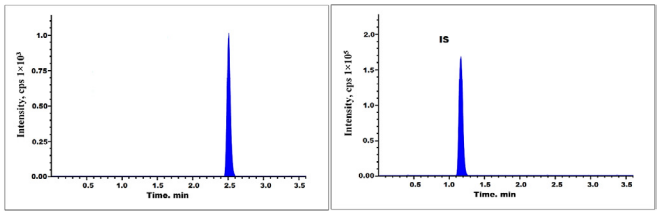

Figure 4: Cabotegravir chromatogram at LQC standard

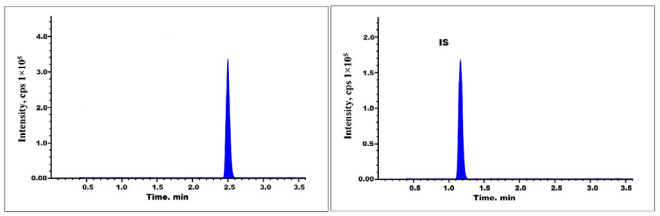

Figure 5: Cabotegravir chromatogram at MQC standard

Table 2: Cabotegravir Precision and Accuracy for Inter-batches and Intra-batches

Concentration level	*Nominal concentration (ng/mL)*	*Intra-batch*			*Inter-batch*		
		Amount found (ng/mL)	*%Accuracy*	*%RSD*	*Amount found (ng/mL)*	*%Accuracy*	*%RSD*
LLoQ	400	385.64	96.41	3.84	415	103.75	5.21
LQC	1120	1061.872	94.81	4.91	1087.072	97.06	2.54
MQC	8000	8298.4	103.73	4.86	8219.2	102.74	3.91
HQC	12000	11445.6	95.38	2.76	11560.8	96.34	4.11

Table 3: Cabotegravir and IS extraction recoveries.

Concentration level	*A*	*B*	*% Recovery*	*% Mean recovery*	*%RSD*
LQC	12689	13050	102.85		
MQC	90640	88682	97.84		
HQC	135960	128169	94.27	98.32	3.58
IS	115324	113202	98.16		

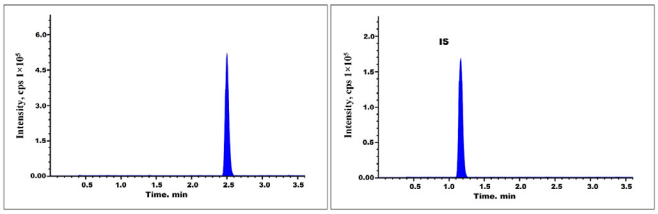

Figure 6: Cabotegravir chromatogram at HQC standard

Table 4: Cabotegravir findings for matrix effect

Cabotegravir	*LQC level*			*HQC level*		
	Analyte MF	*IS MF*	*IS normalized MF*	*Analyte MF*	*IS MF*	*IS normalized MF*
B-1	1.13	1.07	1.05	1.02	1.03	0.99
B -2	1.07	1.04	1.03	1.11	1.07	1.04
B -3	1.1	1.12	0.98	1.1	1.01	1.03
B -4	1.12	1.02	1.05	1.07	1.08	0.99
B -5[x]	1.08	1.06	1.02	1.03	1.02	1.01
B -6[x]	1.04	1.12	0.96	1.09	1.02	1.07
B -7[y]	1.06	1.03	1.03	1.08	1.13	0.97
B -8[y]	1.01	1.05	0.97	1.02	1.09	0.96
Mean	1.016			1		
SD	0.045			0.049		
%RSD	4.46			4.91		

x, Hemolyzed lot; MF, matrix factor; y, Lipemic lot.

Table 5: Cabotegravir stability findings

Parameter	*QC level*	*P*	*Q*	*%RSD*	*%Stability*
Freeze and thaw stability	LQC	1120	1059.956	3.84	94.64
	HQC	12000	11805.31	2.81	98.38
Stability in refrigerator (1–10°C for 48 hours)	LQC	1120	1145.898	4.91	102.31
	HQC	12000	11921.51	3.64	99.34
Bench-top stability (at <10°C for 20 hours)	LQC	1120	1163.592	2.73	103.89
	HQC	12000	11501.55	4.27	95.85
Long term stability (60 days at −20°C)	LQC	1120	1105.987	4.14	98.75
	HQC	12000	12426.16	3.52	103.55
In-injector stability (at 10°C for 72 hours)	LQC	1120	1040.798	3.94	92.93
	HQC	12000	12248.78	4.06	102.07
Long-term stability (60 days at −70°C)	LQC	1120	1089.579	3.28	97.28
	HQC	12000	11733.04	4.27	97.78

Q, mean concentrations (ng/mL); P, nominal concentration (ng/mL) of analytes

48.0 hours, while cabotegravir and IS were unaffected at 1 to 10°C for 70 days. For sixty days, matrix stability was tested at −70 and −20°C. The stability of the matrix was examined using a newly made spiked linear standard. The findings of a stability data were shown in Table 5.[15-18] When the temperature was below 10°C and the drug was subjected to six freeze-thaw cycles, the drug was not destroyed for up to 20 hours. The processed solutions sample were stable for 72 hours at 10°C in the auto-sampler.

Dilution integrity

The ULoQ (upper limit of quantification) for cabotegravir was conducted at double the concentration of the dilution integrity.[13] The mean back-computed drug amount for the dilution tested samples was between 85.0 to 115.0% of the actual amount after the 1:4 dilution, with a %RSD of 3.86.

CONCLUSION

A sensitive and precise LC-MS/MS approach was developed and validated in the current study effort to effectively determine cabotegravir in human plasma. This approach's specificity, accuracy, linearity, precision, and stability were all very high. y = 0.000099x + 0.00335 and 0.9998, respectively, were found to be the linearity equation and correlation coefficient (r2) results. For the QC-samples (400, 1120, 8000, and 12000 ng/mL), the developed technique's percent RSD of intra and inter-day precision ranged from 2.54 to 5.21%. Stability studies were processed at different conditions and the stability values were present in between 92.93% and 103.89%. As a result, the validated approach will be effectively used to conduct pharmacokinetic and toxicokinetic investigations on cabotegravir in various biological matrices for clinical and forensic purposes.

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