**The development and validation of a novel LC-MS/MS method for the quantification of Cenicriviroc in human plasma and cerebrospinal fluid.**

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**Abstract**

A high performance liquid chromatography tandem mass spectrometric method was developed and validated cenicriviroc quantification in human plasma and cerebrospinal fluid. The method involved precipitation with acetonitrile and injecting supernatants onto the column. Separation was achieved on an XBridge C18 column with a gradient elution of 0.1% formic acid in water and acetonitrile. Analyte detection was conducted in positive ion mode using SRM. The m/z transitions were: CVC (697.3→574.3) and CVC-d7 (704.4→574.3). Calibration curve ranged from 5-1000 ng/ml for plasma and 0.241-15.0 ng/ml for CSF. The intra and inter day precision and accuracy were <15% for both plasma and CSF across four different concentrations. Cenicriviroc recovery from plasma and artificial CSF was >90%. The method was utilised for the measurement of patients’ plasma and CSF samples taking a dose of 50, 150 and 300mg qd.

**Keywords: Cenicriviroc, LC-MS/MS, Plasma, CSF, human serum albumin**

**Introduction**

Cenicriviroc (CVC) is a novel potent dual antagonist of the chemokine co-receptors 5 and 2 (CCR5/CCR2) and blocks HIV-1 entry (Kramer, Hassounah, Colby-Germinario, Oliveira, Lefebvre, Mesplede and Wainberg 2015). CVC has been tested in Phase IIb trials, for the treatment of non-alcoholic steatohepatitis in adult subjects with liver fibrosis and consistently demonstrated an improvement in liver fibrosis (Tacke 2018). Cenicriviroc is also expected to have potent anti-inflammatory activity (Lalezari, Gathe, Brinson, Thompson, Cohen, Dejesus, Galindez, Ernst, Martin and Palleja 2011). Furthermore, CVC presents a long half-life (~40hrs) supporting once-daily dosing (Marier, Trinh, Pheng, Palleja and Martin 2011, S. Palleja 2009).

The replication of HIV RNA in the central nervous system (CNS) creates the potential for HIV-associated cognitive impairment. It is postulated that CCR5 antagonists could target HIV RNA replication in sanctuary sites a method previously shown to have beneficial effects in the CNS (Garvey, Nelson, Latch, Erlwein, Allsop, Mitchell, Kaye, Watson, Back, Taylor-Robinson and Winston 2012). This method was developed and validated to assess Cenicriviroc in in plasma and cerebrospinal fluid (CSF) as part of a Phase II feasibility study, (EudraCT 2015-002955-85),

Plasma levels of Cenicriviroc have previously been quantified using liquid chromatography/ tandem mass spectrometry (LC-MS/MS) (Lefebvre, Gottwald, Lasseter, Chang, Willett, Smith, Somasunderam and Utay 2016), but the methods have not been published. Also, to our knowledge, there are no CSF methods published either. We present here a simple tandem mass spectrometric method for quantification of Cenicriviroc in plasma and in CSF using protein precipitation extraction and validated in accordance with EMA and FDA guidelines ( 2012, 2018).

**Materials and Methods**

**Chemicals**

CVC and CVC-d7 were obtained from Allergan Inc, Irvine, California; methanol and acetonitrile (LC-MS grade) were obtained from Sigma–Aldrich. Deionized water (HPLC grade) was obtained from an Elga Option 4 water purifier (Elga LabWater, High Wycombe, United Kingdom). Further purification to 18.2 MΩ was carried out using a Purelab Classic UVF (Elga LabWater). Whole blood, collected in K2EDTA, was obtained from healthy drug-free volunteers with Ethics approval from the NHS Health Research Authority. Artificial CSF was obtained from Harvard Apparatus and Human Serum Albumin from Sigma–Aldrich, UK.

**Equipment**

The chromatographic system was made up of a variable loop Accela autosampler (temperature, 6°C) and an Accela LC pump (Thermo Fisher Scientific, Hemel Hempstead, United Kingdom). The analyte and internal standard (IS) were eluted using a reverse-phase C18 XBridge column (3.5 μm:50 mm × 2.1 mm; Waters UK) with a guard column (at an oven temperature of 40°C. The mass spectrometer was a triple–quadrupole TSQ Quantum Access (Thermo Fisher Scientific, UK) with a heated-electrospray ionization source. Tuning, data acquisition and processing were carried out using TSQ Tune and LC Quan Software (Thermo Fisher Scientific) respectively.

**Stock solution preparation**

Methanolic stock solutions (1 mg/mL) were prepared, with working solutions containing (100 µg/mL and 10 µg/mL of CVC) prepared by diluting the stock solution with more methanol. The internal standard working solutions for plasma (100 ng/mL of CVC-d7) and CSF (10 ng/mL of CVC-d7) were also prepared in methanol.

For plasma, working calibration sub-stock solutions were prepared from 100µg/mL by diluting in the appropriate volume of methanol to a final concentration of 50, 12.5 and 0.625 µg/ml. Working calibration standards were prepared by spiking blank human plasma to yield 12.5, 250, and 1000 ng/mL CVC calibration standard solutions. The working calibration standard solutions were serially diluted to yield final plasma concentrations of 5.0, 12.5, 50.0, 125.0, 250.0, 500.0, 800.0, and 1000.0 ng/mL of CVC. All standards were prepared using calibrated air-displacement pipettes.

For CSF, working calibration sub-stock solutions were prepared from 10 µg/mL by diluting in the appropriate volume of methanol to a final concentration of 0.012, 0.019, 0.049, 0.097, 0.195, 0.433, 0.623 and 0.750 µg/mL. Calibration curves were prepared by spiking the working calibration sub-stock solution into artificial CSF containing 0.5% human serum albumin, yielding eight calibration standards (0.241, 0.389, 0.973, 1.947, 3.894, 8.653, 12.45, 15.0ng/mL). Quality Control (QC) samples were prepared from the CVC QC primary stock. These consisted of High QC (750 ng/mL), Medium QC (150 ng/mL), Low QC (11.3 ng/mL; 3x the lowest calibration standard concentration) and the lower limit of quantification (LLQ; 5.0 ng/mL) for plasma and for CSF (HQC; 12.50, MQC; 2.25, LQC; 0.720 and LLQ; 0.241 ng/mL).

**Non-specific binding of Cenicriviroc in CSF**

Hydrophobic compounds are often lost due to adsorption onto glass and plastic lab-ware when in low-protein biological matrices such as urine and CSF (Gu, Deng, Wang, Aubry and Arnold 2010, Ji, Jiang, Livson, Davis, Chu and Weng 2010, P.Nouri 2016). Minimising or resolving the adsorption loss is often achieved using a variety of methods including: addition of plasma, bovine serum albumin or surfactants such as Tween 20, sodium dodecylbenzenesulfonate or 3-[3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS)(Chen, Bajpai, Mollova and Leung 2009, Silvester and Zang 2012, Xu, Du, Rose, Fu, Woolf and Musson 2005)

There was a significant loss of Cenicriviroc observed in aCSF, with increased variability from the nominal concentrations (%Bias) and within replicates. This loss was observed across the entire calibration range from 0.24 ng/mL to 15 ng/mL. This was due to non-specific binding of Cenicriviroc to the glass tubes when serial dilutions were performed in CSF. Separate preparation of individual calibrant levels in pure methanol, to eliminate the serial dilution step, followed by direct spiking into blank CSF did not reduce this effect.

The addition of human serum albumin at different concentrations (0.01-1%) was evaluated as a method of preventing the non-specific binding of CVC in CSF samples. Based on the results, artificial CSF was pre-treated with 0.5% human serum albumin before the preparation of calibration standards and quality control samples. (Table 1).

The addition of 0.5% human serum albumin (w/v) greatly improved analyte response and decreased the variability.

**TABLE 1**

**Sample Preparation**

CVC was extracted from both plasma and CSF by protein precipitation using acetonitrile. Standards, QCs, blank and study samples (100µl) were aliquoted into 5mL glass test tubes, to each of which internal standard working solution (CVC-d7; 20µl) was added. 100µl of 0.1% formic acid solution in water was also added to the mixture followed by precipitation with 500µl of acetonitrile. Samples were vortexed for approximately 1 minute and then centrifuged (4000 rpm, 4°C, 5 minutes) and the supernatant transferred to autosampler vials. The vials were loaded onto autosampler trays and 5μL injected into the LC-MS/MS system for analysis.

**LC-MS/MS Procedure**

Chromatographic separation was achieved using a Waters C18 XBridge column (3.5μm: 50 mm × 2.1 mm) and 5mM ammonium acetate solution with 0.1% formic acid (mobile phase A) and acetonitrile (mobile phase B). Cenicriviroc was eluted from the column using a gradient method at a flow rate of 400µl/min. Mobile phase gradient started with 80% mobile phase A, which was held for 0.3 minutes then increasing in organic content to 85% mobile phase B in 0.8 minutes. This was maintained up to 2.5 minutes followed by increasing the organic content to 100% for 1 minute and back to the initial conditions for reconditioning with a total run time of 5 minutes.

The electrospray ionisation (ESI) mass spectrometer (MS) was operated in positive ion mode using selective reaction monitoring (SRM). These were the MS settings:- electrospray voltage, 5.0kV; capillary temperature, 270°C; vaporiser temperature, 350°C; sheath and auxiliary gas pressures, 50 and 20 arbitrary units respectively. The collision gas Argon, was delivered at a pressure of 1.5mTorr with collision energy set to 20 and tube lens set to 50. The m/z transitions for CVC was 697.3→574.3 and CVC-d7, 704.4→574.3 and. The scan width was set at 0.01 and the scan time at 0.1 seconds. The peak width settings for Q1 and Q3 were set at unit resolution (0.7).

Initial chromatographic optimisation was with a conventional C18 column using acetonitrile and formic acid as additives at low concentrations in mobile phase but this resulted in peak tailing. Altering the pH and buffers at to different concentrations did not improve the peak shape. Cenicriviroc is a weak polyprotic base (pKa=6.39), making the molecule prone to secondary interaction with silanes of the column. Changing the column to a high purity silica type with end-capping was considered, in order improve the peak shape. The XBridge C18 column is known for its Ethylene Bridged Hybrid (BEH) technology and advanced end-capping. The previously used mobile phase was not sufficient to get a reasonable signal and a good peak shape using the XBridge column. However, addition of 5mM ammonium acetate to water containing 0.1% formic acid resulted in sharper peaks with excellent sensitivity.

**Validation methodology**

**Selectivity**

Six different lots of blank human plasma were used in this assessment. Selectivity was determined at the LLQ, where the precision should be ≤20% and accuracy within 20% of the nominal concentrations. (n = 6). Also, interference or noise response at the same retention time of internal standard were deemed acceptable if the % interference was less than 5% of the mean response of the internal standard areas in 6 LLQ samples.

**Accuracy and Precision**

Three separate accuracy and precision batches were run consisting of a calibration curve and LLQ, LQC, MQC and HQC samples in replicates of six. Data from the manufacturers’ observed Cmax following standard dosing (and adjusted dosing based on interaction with other ART agents that induce or inhibit hepatic iso-enzymes) served as a guide to setting the calibration range.

**Carryover**

The carryover was assessed by injecting blank samples, followed by assay LLQ (5 ng/mL) and upper limit of quantification (ULQ; 1000 ng/mL) standards, run in duplicate. This was followed by 3 blank plasma samples. The % carryover (in the blank samples after ULQ) was calculated and expressed in relation to the assay LLQ; the % carryover should not exceed 20% of the LLQ concentration (EMA Bioanalytical method validation) .

**Dilution integrity**

CVC concentrations between 160-180% of the assay ULQ was spiked into plasma, which was subsequently diluted 1:2 and 1:4 with blank plasma. The samples were then analysed, with concentrations from the standard curve (including the appropriate dilution factor), and compared against the expected nominal concentration.

**Recovery and Matrix effects**

The % recovery and matrix effects were determined quantitatively using the methods of Matuszewski *et al* (Matuszewski, Constanzer and Chavez-Eng 2003). The % recovery (process efficiency) was obtained by comparing the peak-area of the analyte at LQC/MQC/HQC concentrations from extracted plasma samples, to the peak area of analyte spiked at an equivalent concentration in mobile phase. The % matrix effect compared the peak areas of CVC spiked into blank plasma extracts with the peak areas of CVC in mobile phase at an equivalent concentration.

**Stability**

The stability of Cenicriviroc in plasma and CSF under different conditions (QC samples; 6 per level) was assessed after:- heat inactivation at 58oC for 40 minutes; and over 3 freeze-thaw cycles spanning a period of 3 days. Bench-top stability was also assessed at room temperature over 6 hours (n=4, per level); and furthermore, by re-injecting an accepted precision and accuracy batch (6 QC per level) which had been left in the autosampler at 4oC for 48 hours, reinjection reproducibility was assessed.

**Measurement of Cenicriviroc in human plasma and CSF**

Blood samples for the pharmacokinetic analysis of CVC in a phase II open-label feasibility study in 7 HIV-positive volunteers were analysed using this method. Of the seven participants, only four completed all study procedures (EudraCT 2015-002955-85). The study was conducted at St Mary’s Hospital, Imperial College Healthcare NHS Trust, London, UK, between March and December 2016. Human ethics approval was obtained (The Brighton and Sussex Research Ethics Committee, UK, reference: 15-LOC-1887). Cenicriviroc was administered once daily in the morning with food, for 8 weeks. Dose was 150 mg daily when administered with ART without significant effects on hepatic isoenzymes. This dose was reduced to 50 mg daily or increased to 300 mg daily when administered alongside ART with significant inhibition effects (HIV protease inhibitors) or with significant induction effects (efavirenz) on hepatic isoenzymes, respectively. Blood was drawn at baseline and week 8. Collected whole blood was immediately placed on ice until centrifugation to separate plasma from the blood cells. Plasma was aliquoted and stored at −40 °C until analysis. The subjects had their CSF collected by lumbar puncture. The CSF samples were aliquoted and immediately placed at−40 °C freezer until analysis.

**Method Validation**

**Selectivity**

There was minimal background interference (<10% of the signal response at the LLQ for CVC) and suitable selectivity in all six plasma batches chosen.

**Accuracy and Precision**

The accuracy and precision (both inter- and intra-assay) values at the assay LLQ fell within the designated ±20% and were within ±15% of the nominal values for all QC levels. (**Table 2)**.

**TABLE 2**

**Carryover**

The mean % carryover (n=3) observed in the first extracted blank sample following injection of an ULQ sample (1000 ng/mL) was 0.13% for CVC. This represented 26.9% of the assay LLQ that, upon injection of the second blank plasma sample, reduced to 0.03% for CVC which is equivalent to 5.8% of the assay LLQ.

**Dilution integrity**

Samples diluted by a factor of 2 and 4 times showed calculated concentrations within ± 15% of the nominal values. The %CV for the diluted samples was <10% for Cenicriviroc.

**Recovery and Matrix effects**

Matrix effects (ME), Recovery Efficiency (RE), and Process Efficiency (PE) were studied for both plasma and CSF matrices each at three different concentrations. Overall recovery is >90% in both plasma and CSF with negligible matrix effect; the data can be seen in **Table 3**.

**TABLE 3**

**Stability**

Stability data are presented in **Table 4**. Samples were stable for up to 48 hours following re-injection within the LC-MS/MS autosampler (4oC) with concentrations within ±15% of the respective nominal values. Cenicriviroc was also stable after heat treatment and through 3 freeze-thaw cycles (n=4). Furthermore, when left on the bench at room temperature, the samples were stable for up to 6 hours.

**TABLE 4**

**Application of method to clinical pharmacokinetic study**

All subjects had plasma and cerebrospinal fluid cenicriviroc concentrations below the LLQ at baseline. At week eight, peak plasma cenicriviroc concentrations was detectable in all four subjects, but detectable in the cerebrospinal fluid in only two subjects (mean 0.82 and 0.40 ng/mL respectively), and below the LLQ in the other two subjects. Mean cerebrospinal fluid: plasma cenicriviroc concentration ratio was no more than 0.18% (95% CI of the upper estimate 0.09% – 0.28%).(Alagaratnam, Dilly-Penchala, Challenger, Else, Legg, Petersen, Jones, Kulasegaram, Seyedkazemi, Lefebvre, Khoo and Winston).

**TABLE 5**

**Conclusion**

In this study, a sensitive, selective, accurate and robust LC–MS/MS method was developed and validated to quantify cenicriviroc in human plasma and CSF. This, to the best of our knowledge, is the first method quantifying cenicriviroc concentrations in CSF.

Furthermore, this assay will provide a greater understanding of CVC pharmacokinetics across different individuals under different treatment scenarios. The incurred sample analysis met the acceptance criteria and as previously mentioned, the method has been used to study the pharmacokinetics of cenicriviroc in plasma and CSF as part of a clinical trials.

**Acknowledgments**

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**Legends**

**Figure 1.** Chromatograms of cenicriviroc and cenicriviroc-d7 in **CSF** in a) Blank aCSF sample b) Spiked with 0.24ng/ml (LOQ) c) Patient sample at week 8 d) Internal standard CVC-d7

**Figure 2.** Chromatograms of cenicriviroc and cenicriviroc-d7 in **Plasma** e) Chromatogram of Blank plasma sample f) Spiked with 5ng/ml (LOQ) g) Patient sample at week 8 h) Internal standard CVC-d7

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**Table 1.** Variability from nominal concentrations (%Bias) observed before and after pre-treatment with human serum albumin (0.5% w/v)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Nominal Concentration**  **(ng/mL)** | **Untreated artificial CSF** | | **Artificial CSF pre-treated**  **with 0.5% serum albumin human** | |
| **Duplicate back calculated concentration (ng/mL)** | **%Bias** | **Duplicate back calculated concentration (ng/mL)** | **%Bias** |
| 0.241 | 0.309 | 28.2 | 0.200 | -17.0 |
| 0.277 | 14.9 | 0.280 | 16.0 |
| 0.389 | 0.336 | -13.6 | 0.416 | 7.0 |
| 0.347 | -10.8 | 0.393 | 1.1 |
| 0.973 | 0.453 | -53.4 | 0.898 | -7.7 |
| 0.548 | -43.7 | 0.922 | -5.2 |
| 1.947 | 1.292 | -33.6 | 1.714 | -12.0 |
| 0.809 | -58.4 | 1.821 | -6.5 |
| 3.894 | 6.402 | 64.4 | 4.098 | 5.3 |
| 4.681 | 20.2 | 3.699 | -5.0 |
| 8.653 | 9.673 | 11.8 | 8.118 | -6.2 |
| 11.139 | 28.7 | 8.317 | -3.9 |
| 12.450 | 14.520 | 16.6 | 15.043 | 20.8 |
| 16.623 | 33.5 | 14.053 | 12.9 |
| 15.000 | 14.326 | -4.5 | 16.535 | 10.2 |
| 14.931 | -0.5 | 15.884 | 5.9 |

**Table 2.** Accuracy & Precision (inter- and intra-day) for CVC in Plasma & CSF

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Plasma** | **LLQ (5 ng/mL)** | | | | **LQC (11.3 ng/mL)** | | | | **MQC (150 ng/mL)** | | | | **HQC (750ng/mL)** | | | | |
| **Mean** | **SD** | **%CV** | **%Bias** | **Mean** | **SD** | **%CV** | **%Bias** | **Mean** | **SD** | **%CV** | **%Bias** | **Mean** | **SD** | **%CV** | **%Bias** |
| **Inter-day** | 5.23 | 0.54 | 10.26 | 4.59 | 11.90 | 0.82 | 6.90 | 5.33 | 159.52 | 12.96 | 8.12 | 6.35 | 792.53 | 54.68 | 6.90 | 5.67 |
| **Intra-day** | 5.46 | 0.305 | 5.59 | 9.21 | 12.38 | 0.51 | 4.15 | 9.58 | 165.01 | 4.66 | 2.83 | 10.00 | 789.03 | 33.90 | 4.30 | 5.20 |
|  | | | | | | | | | | | | | | | | | |
| **CSF** | **LLQ (0.241 ng/mL)** | | | | **LQC (0.720 ng/mL)** | | | | **MQC (2.25 ng/mL)** | | | | **HQC (12.5 ng/mL)** | | | | |
| **Mean** | **SD** | **%CV** | **%Bias** | **Mean** | **SD** | **%CV** | **%Bias** | **Mean** | **SD** | **%CV** | **%Bias** | **Mean** | **SD** | **%CV** | **%Bias** |
| **Inter-day** | 0.26 | 0.04 | 13.70 | 9.42 | 0.72 | 0.072 | 10.05 | -0.59 | 2.36 | 0.32 | 13.51 | 3.62 | 12.48 | 1.78 | 14.25 | -1.04 |
| **Intra-day** | 0.25 | 0.04 | 14.74 | 4.54 | 0.74 | 0.07 | 9.63 | 2.36 | 2.42 | 0.15 | 6.26 | 8.71 | 13.57 | 1.42 | 10.49 | 8.60 |

**CV - Coefficient of Variation; SD – Standard Deviation; LQC – Low Quality Control samples; MQC – Medium Low Quality Control samples; HQC – High Low Quality Control samples**

**Table 3.** Recovery & matrix effect of Cenicriviroc in plasma and CSF

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Matrix** | **Nominal QC concentration in ng/mL**  **(L, M, H)** | **Mean peak area**  **A(n=6) B(n=6) C(n=6)** | | | **Mean peak Area ratio**  **B2 C2** | | **ME (%) B/A** | **Ext RE (%)**  **C/B** | **PE (%) C/A** | **Analysis RE (%) C2/B2** |
| **Plasma** | 11.3 | 152234 | 155868 | 149635 | 0.39 | 0.41 | 102.4 | 96.0 | 98.3 | 105.1 |
| 150 | 1896308 | 1842656 | 1891241 | 6.3 | 5.83 | 97.2 | 102.6 | 99.7 | 92.5 |
| 750 | 8873971 | 8749945 | 8816279 | 31.47 | 28.41 | 98.6 | 100.8 | 99.3 | 90.3 |
|  | | | | | | | | | | |
| **CSF** | 0.72 | 6171 | 6684 | 5801 | 0.40 | 0.44 | 108.3 | 86.8 | 94.0 | 109.8 |
| 2.25 | 27740 | 28987 | 31494 | 1.30 | 1.41 | 104.5 | 108.6 | 113.5 | 108.5 |
| 12.5 | 163512 | 149438 | 143166 | 12.72 | 11.55 | 91.4 | 95.8 | 87.6 | 90.8 |

**ME (%) - % Matrix effect; Ext RE (%) - % Extraction Recovery Efficiency; PE (%) - % Process Efficiency; Analysis RE (%) - % Analysis Recovery Efficiency; L – Low, M - Medium, H - High**

**Table 4.** Stability data for CVC under different storage conditions

|  |  |  |  |
| --- | --- | --- | --- |
| **Stability measured** | **QC** | **Precision (%CV)** | **Accuracy (%)** |
| **Benchtop (6h)** | LQC | 10.08 | -9.69 |
| MQC | 5.16 | 1.22 |
| HQC | 10.81 | -1.09 |
| **Heat inactivation** | LQC | 6.56 | -3.47 |
| MQC | 1.41 | 7.61 |
| HQC | 4.24 | 0.679 |
| **Autosampler (48h)** | LQC | 1.79 | -8.875 |
| MQC | 5.57 | 1.016 |
| HQC | 1.01 | -1.186 |
| **Reinjection reproducibility (48h)** | LQC | 7.10 | 1.424 |
| MQC | 4.94 | 4.614 |
| HQC | 8.35 | 1.55 |

**QC – Quality Control samples; LQC – Low Quality Control samples; MQC – Medium Low Quality Control samples;**

**HQC – High Low Quality Control samples**

**Table 5.** Individual subject blood and cerebrospinal fluid concentration at week 8

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Cenicriviroc concentration** | **Subject 1** | **Subject 2** | **Subject 3** | **Subject 4** |
| **CSF, ng/mL** | 0.82 | 0.40 | (<LLQ) | (<LLQ) |
| **Plasma, ng/mL** | 718.60 | 211.06 | 411.93 | 70.50 |
| **CSF: plasma cenicriviroc concentration (%)** | 0.11 | 0.19 | --- | --- |
| **Antiretroviral therapy** | abacavir,  lamivudine,  raltegravir | lamivudine, atazanavir, ritonavir | tenofovir DF, emtricitabine, dolutegravir | tenofovir DF, emtricitabine, raltegravir |
| **Cenicriviroc dose** | 150 mg | 50 mg | 150 mg | 150 mg |

**LIST OF FIGURES**

**Figure 1.** Chromatograms of cenicriviroc and cenicriviroc-d7 in **CSF**

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|  |
| a) |
|  |
| b) |
|  |
| c) |
|  |
| d) |

**Figure 2.** Chromatograms of cenicriviroc and cenicriviroc-d7 in **Plasma**

|  |
| --- |
|  |
| e) |
|  |
| f) |
|  |
| g) |
|  |
| h) |