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| **Protocol Reference** | PRO MV 0138-1, Effective: May 11, 2021 |
| **Notebook References** | |  |  | | --- | --- | | **Notebooks** | **Pages** | | ARD-0307 | 7-17, 22-25 | | ARD-0247 | 64-102 | | ARD-0270 | 17-23, 30-45 | |
| **Analysts** | Ran Li  Lin Sun  Marjorie Cordero |

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# Introduction

CX-4945 Sodium Salt (Formula: C19H11ClN3O2Na; molecular weight: 371.75 g/mol) is chemically known sodium 5-(3-chlorophenylamino)benzo[c][2,6]naphthyridine-8-carboxylate. The structural formula of CX-4945 is represented below:



This report summarizes the findings from execution of PRO MV 0138-1, which pertains to the method verification by Frontida BioPharm ARD department of the *Dissolution* analytical procedure for CX-4945 (Silmitasertib) Capsules, 200 mg.

The method qualification of analytical procedure has been previously successfully performed by Alcami, the findings from which are summarized in corresponding method qualification report provided by Senhwa Biosciences, Inc. (Report#: RPT 71219.00). The target dissolution limit is shown in Table 1-1.

**Table 1-1.** Target Limit for CX-4945 Capsules (200 mg)

|  |  |
| --- | --- |
| **Test** | **Target Limit** |
| Dissolution | NLT 80% (Q) of the labeled amount of CX-4945 is dissolved in 45 minutes |

The qualification of the method included and demonstrated the following method parameters/characteristics:

* System Suitability
* Specificity (Interference)
* Linearity
* Method Repeatability
* Filter Study
* Stability of Solutions

A modification was made from Alcami’s method to the detection wavelength from 265 nm to 360 nm.

Appropriate verification studies were performed by the Frontida BioPharm ARD department in order to verify the suitability of the method and demonstrate the capability of the laboratory to perform the analysis. The results were assessed as defined in the method verification protocol. Changes or deviations from the protocol are reflected in this report.

The studies were performed in accordance with Frontida BioPharm’s Standard Operating Procedure (SOP) for Validation of Analytical Methods, SOP MPC QC/RD-017 (current version).

The following studies were performed for suitability of test method and demonstrate the capability of Frontida BioPharm ARD department to perform this analysis:

* System Suitability
* Specificity (Interference)
* Linearity and Range
* Accuracy
* Precision (Repeatability)
* Filtration Study
* Stability of Solutions

# Analytical Procedure

The below section describes the final analytical procedure performed for method validation and has been updated to include changes or deviations, if any, from those described in the corresponding section in the method validation protocol.

## Chromatographic Parameters

**Table 2-1.** HPLC Parameters

|  |  |
| --- | --- |
| **HPLC System** | Waters Acquity HClass |
| **Column** | Agilent InfinityLab Poroshell 120 EC-C18, 50 x 4.6 mm, 2.7 μm  Part Number: 699975-902 |
| **Mobile Phase A** | 0.1% TFA in Purified Water |
| **Mobile Phase B** | 0.05% TFA in Acetonitrile |
| **Needle Wash** | 50:50 Acetonitrile: Purified Water |
| **Gradient Program** | |  |  |  | | --- | --- | --- | | Time (min) | A (%) | B (%) | | 0 | 90 | 10 | | 6.0 | 10 | 90 | | 6.1 | 90 | 10 | | 9.0 | 90 | 10 | |
| **Detection** | 360 nm |
| **Flow Rate** | 1.0 mL/min |
| **Column Temperature** | 40°C ± 5°C |
| **Autosampler Temperature** | 5°C ± 3°C |
| **Injection Volume** | 2 μL |
| **Sampling Rate** | 10 points/sec |
| **Run Time** | 9 minutes |

## Dissolution Conditions

Table 2-2. Dissolution Conditions

|  |  |
| --- | --- |
| **Medium** | Purified Water |
| **Volume** | 900 mL |
| **Apparatus** | USP Type II (Paddles) |
| **Speed** | 50 RPM |
| **Time** | For single pull: 45 minutes  For profile: 5, 15, 30, 45, 60 minutes (infinity: 60 min at 250 rpm) |
| **Temperature** | 37.0°C ± 0.5°C |
| **Pull Volume** | 10 mL |
| **Filter** | 0.45 µm PVDF filter |
| **Sinker** | Wire Helix |

## Reagents and Materials

* Purified Water, Millipore
* Acetonitrile (ACN), HPLC Grade
* Trifluoroacetic Acid (TFA), HPLC Grade
* CX-4945 (free acid) Standard, client provided
* CX-4945 Capsules composite placebo
* Whatman 0.45-μm PVDF membrane filter

## Dissolution Medium Preparation

Transfer 6000 mL of purified water into a suitable flask. Mix well and degas.

## Mobile Phase A Preparation (0.1% TFA in water)

Transfer 1.0 mL of TFA into a suitable flask containing 1000 mL of purified water. Mix well.

## Mobile Phase B Preparation (0.05% TFA in Acetonitrile)

Transfer 0.5 mL of TFA into a suitable flask containing 1000 mL of acetonitrile. Mix well.

## Diluent Preparation

Transfer 50 mL of TFA into a suitable flask containing 950 mL of acetonitrile. Mix well.

## Standard Solution Preparation

*Standard Usage Note: Prior to use, standard must be ground with a mortar and pestle and then equilibrated to ambient laboratory conditions for at least one hour, but not more than 2 hours.*

*Determine the water content of the ground, equilibrated standard on the day of use as per current USP <921> Method Ia (performed as per SOP MPC RD 065, SOP MPC RD 066; SOP MPC QC 197, SOP MPC QC 198) as follows:*

*Diluent: Methanol Dry*

*Titrant: Composite 2*

*Sample Amount: About 100 mg (or adjusted as needed to obtain an amount of water between 2 mg to 250 mg)*

*Perform the water determination in duplicate. The absolute difference between the two results should be NMT 1.0%. Report the mean of two determinations.*

Accurately weigh the equivalent of approximately 22 mg of CX-4945 free acid standard by quantitatively transferring into a 100-mL volumetric flask an amount (in mg) of standard adjusted for its purity as follows:

, where *P* is the purity of reference standard expressed as % Purity/100%. Add about ¾ volume of diluent and mix to dissolve. Sonicate to dissolve if necessary. Allow solution to cool to room temperature, then dilute to volume with diluent and mix well.

The concentration of CX-4945 free acid is 0.22 mg/mL.

Prepare a check standard solution in a similar manner.

## Sample Solution Preparation

At each dissolution analysis time point, withdraw a 10 mL portion of the solution at a zone midway between the surface of the dissolution medium and the top of the rotating paddle and not less than 1 cm from the vessel wall. Filter the solution through a Whatman PVDF 0.45-µm filter, discarding the first 3 mL of the filtrate.

Note—Dispense sample solutions directly into HPLC vials for analysis. The results obtained at all other profile time points besides at endpoint (45 minutes) are only for reporting purposes and will not appear in the finished product Certificate of Analysis.

## Procedure

Separately inject equal volumes (2 µL) of the dissolution media, diluent, standard, and sample solutions. Record the chromatograms and measure the peak area responses of the CX-4945 peak.

A maximum of six injections of sample may be performed between bracketing standard injections

## System Suitability Requirements

* The diluent and dissolution media injections should have no peaks that elutes at RRT 0.98 – 1.02 of the CX-4945 peak, which significantly interfere (NMT 1% relative to the average peak area of the CX-4945 peak from the five replicate injections of working standard) with the quantitation of CX-4945
* The % RSD of the CX-4945 retention time from the five (5) consecutive injections of working standard solution is NMT 2.0%.
* The % RSD of the CX-4945 peak area responses from the five (5) consecutive injections of working standard solution is NMT 2.0%.
* The mean USP Tailing Factor (Tf) for the CX-4945 peak from the five (5) consecutive injections of working standard solution is NMT 2.0.
* Standard check agreement should be between 98.0 – 102.0%.

## Calculations

Calculate the % dissolved as follows:

For Single Time Point and 1st Time Point of Profile:

For All Other Profile Time Points:

Where,

Rspl : The area response of CX-4945 in the sample solution

Rs : The area response of CX-4945 in the standard solution

Ws : Weight of the CX-4945 free acid standard, in mg

P : Purity of the CX-4945 free acid standard expressed as % Purity/100%

LC : Nominal Label Claim of CX-4945 Capsules, in mg

V : Withdraw Volume, in mL

n : Sampling time point number

# Instruments/Equipment and Reagents/MateriaLs

## Instruments and Equipment:

* Waters Acquity H-Class (Instrument: ARD UPLC07, Calibration Due: 03/22)
* Sartorius Analytical Balances (Balance: ARD AB13, Calibration Due: 08/21; Balance: ARD AB18, Calibration Due: 07/21)
* Agilent InfinityLab Poroshell 120 EC-C18, 50 x 4.6 mm, 2.7 μm, Part Number: 699975-902, S/N: USCFU39671

## Reagents and Materials

* Purified Water, Millipore, In-house
* Acetonitrile (ACN), Mfr.: OmniSolv, Lot# 60358, Exp. Date: 03/24, Storage: RT
* Trifluoroacetic acid (TFA), Mfr.: Sigma Aldrich, Lot# MKCL3567, Exp. Date: 10/21, Storage: RT
* CX-4945 standard, Mfr.: Carbogen Amcis AG, Lot# NE-023568-A-1-7 Crude 2#1, Exp. Date: 05/22, Storage: RT, Purity: 93.9583%
* CX-4945 sodium salt drug substance, Mfr.: Carbogen Amcis AG, Lot# CA17-0654, Exp. Date: 01/21, Storage: RT, Purity: 83.062%
* CX-4945 Capsules, 200 mg, Mfr.: Senhwa Biosciences, Lot# B180393, Storage: RT
* CX-4945 Capsules composite placebo, Lot# NB071:002, Storage: RT
* Empty hard gelatin capsules, Mfr.: Capsugel, Lot# RL00311, Storage: RT
* Whatman PVDF 0.45-µm membrane filter, Lot# 6872-2504

# system suitability

The System Suitability of the test method was performed and demonstrated as part of establishing system suitability for the subsequent verification studies. The successful establishment of the system suitability requirements (as described in **Section 2.11**) is considered fulfillment of this study.

# Specificity (Interference and Identification)

## Dissolution medium Interference Solution Preparation

The *Dissolution medium* was used as the dissolution medium interference solution

## Diluent Interference Solution Preparation

The *Diluent* was used as the diluent interference solution.

## 2X Placebo Interference Solution Preparation

About 252 mg of CX-4945 capsule composite placebo and one (1) empty capsule was transferred into a 900-mL volumetric flask. Dissolution media, previously preheated to about 37ºC, was added to fill about ¾ of the flask volume. The solution was stirred for 45 minutes, upon which stir bar was removed, diluted to volume with dissolution media and mixed well. A portion of the solution was filtered through a 0.45‑μm Whatman PVDF membrane filter, discarding the first 3 mL of the filtrate.

## Results and Discussion

All system suitability requirements were met.

There were no significantly interfering peaks (NMT 1% of CX-4945 from working standard injections) presented at the retention time of CX-4945 peak from injections of the diluent interference, dissolution medium interference, and 2x placebo interference solutions.

**Figure 1** is a representative chromatogram of the working standard solution.

**Figure 2** is a representative chromatogram of the dissolution medium interference solution.

**Figure 3** is a representative chromatogram of the diluent interference solution.

**Figure 4** is a representative chromatogram of the 2x placebo interference solution.

# Linearity and Range

The linearity study was evaluated from about 5% to 120% of the CX-4945 concentration of the working standard solution, which corresponded to about 11 µg/mL to 264 µg/mL.

## Stock Linearity Solution

Approximately 58.5 mg of CX-4945 free acid standard was accurately weighed and quantitatively transferred into a 50‑mL volumetric flask. Diluent was added to about ¾ of the flask volume. Solution was sonicated to dissolve the material. Solution was cooled to room temperature, diluted to volume with the diluent and mixed well.

The concentration of CX-4945 free acid was about 1.1 mg/mL.

## Working Linearity Solution Preparations

Working linearity solutions were prepared as directed in Table 6-1. Each solution was diluted to volume with diluent and mixed well.

Table 6-1. Preparation of working linearity solutions

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Linearity  Level | Nominal Conc.  (%) | Volume of Stock Linearity Solution  (mL) | Volume of Working Linearity L5 Solution (mL) | Flask Volume (mL) | Approx. Conc.  CX-4945  (µg/mL) |
| L1 | 5 | — | 5.0 | 100 | 11 |
| L2 | 25 | — | 12.5 | 50 | 55 |
| L3 | 50 | 5.0 | — | 50 | 110 |
| L4 | 75 | 7.5 | — | 50 | 165 |
| L5 | 100 | 10.0 | — | 50 | 220 |
| L6 | 120 | 12.0 | — | 50 | 264 |

## Results and Discussion

All system suitability requirements were met.

The linearity results are summarized in **Table 6-2**. All criteria were met.

The peak areas were plotted against their corresponding concentrations and a linear regression analysis was performed. **Figure 5** is the plot of the area response vs. concentration. **Figure 6** is the plot of the relative response factors vs. concentration. The y-intercept relative to the peak area response at the L5-100% level was 0.2%, which was not significantly different from zero.

The linearity was demonstrated from about 11 µg/mL to 264 µg/mL, which corresponds to about 5% to 120% of the nominal working standard concentration.

Table 6-2: Linearity Results

(Notebook Reference: ARD-0307, pg. 15)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Level** | **Nominal Conc.  (%)** | **Concentration (µg/mL)** | **Area Response** | **Response Factor** | **Relative Response Factor (RRF), %** |
| L1 | 5 | 11.012745 | 34349.2297 | 3119.0434 | 100.8 |
| L2 | 25 | 55.063725 | 171331.5348 | 3111.5137 | 100.5 |
| L3 | 50 | 110.12745 | 340310.7497 | 3090.1537 | 99.9 |
| L4 | 75 | 165.191175 | 511058.0824 | 3093.7371 | 100.0 |
| L5 | 100 | 220.25490 | 681598.9031 | 3094.5913 | 100.0 |
| L6 | 120 | 264.30588 | 813071.7620 | 3076.2530 | 99.4 |
| **Slope** | | | | 3079.4481 | |
| **Y-Intercept** | | | | 1371.9889 | |
| **% Y-Intercept Relative to Target 100% Level** | | | | 0.2 | |
| **Correlation Coefficient, R** | | | | 1.000 | |
| Acceptance Criteria:  • Meet the linearity range of a minimum of five consecutive levels.  • The relative response factors (RRF) at each level is within 97.0% to 103.0%.  • The correlation coefficient, R, is NLT 0.995.  • The percent y intercept is NMT 2%. | | | | | |

# Accuracy by “Spiked” recovery study

Accuracy study was performed in order to demonstrate that the method can achieve acceptable recoveries.

The accuracy study was performed by spiking CX-4945 drug substance solution into an amount of composite placebo corresponding to the 200 mg dosage strength. The accuracy was evaluated from about 5% to 150% of the CX-4945 concentration of the working standard solution, which corresponds to about 11 µg/mL to 330 µg/mL of the CX-4945. In order to exclude effects of potency differences using the drug substance in the sodium salt form, the recovery was calculated against a control prepared using the CX-4945 sodium salt drug substance.

## Recovery Solution Preparation

For each recovery level, triplicate sample solutions were prepared.

Perform the dissolution of CX-4945 sodium salt drug substance, composite placebo, and one (1) empty capsule shell as per **Section 2.2**.

Table 7-1. Preparation of recovery sample solutions.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Recovery Level | Nominal Conc.  (%) | Weight of CX-4945 Sodium Salt  (mg) | Weight of Composite Placebo  (mg) | Quantity of Empty Capsule | Volume Dissolution Media (mL) | Approx. Conc.  CX-4945  (µg/mL) |
| R1 | 5 | 10 | 140 | 1 | 200 | 11 |
| R2 | 50 | 100 | 140 | 1 | 200 | 110 |
| R3 | 100 | 200 | 140 | 1 | 200 | 220 |
| R4 | 150 | 300 | 140 | 1 | 200 | 330 |

## Control Solution Preparation

Triplicate sample solutions were prepared.

Perform the dissolution as per Section 2.2 of CX-4945 sodium salt drug substance.

## Results and Discussion

All system suitability requirements were met.

The recovery results are summarized in **Table 7-2**. All criteria were met.

Accuracy of the method was demonstrated from about 11 µg/mL to 330 µg/mL, which corresponds to 5% to 150% of the specification.

Table 7-2. Recovery Results

(Notebook Reference: ARD-0247, pg. 99-100)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Recovery Sample Level** | **Nominal Concentration, (%)** | **Recovery, (%)** | **Mean Recovery, (%)** | **RSD, (%)** |
| R1 | 5 | 102.62 | 102 | 0.4 |
| 102.02 |
| 101.78 |
| R2 | 50 | 102.52 | 102 | 0.1 |
| 102.56 |
| 102.29 |
| R3 | 100 | 100.90 | 100 | 0.9 |
| 99.60 |
| 99.08 |
| R4 | 150 | 99.65 | 100 | 0.2 |
| 99.26 |
| 99.64 |
| Validity/Acceptance Criteria:  • Meet the system suitability requirements in Section 2.11.  • The percent RSD of results for levels R1 is NMT 6.0%.  • The percent RSD of results for levels R2 to R4 is NMT 3.0%.  • The mean percent recovery of the R1 level is within 90%-110%.  • The mean percent recovery of the R2 to R4 levels is within 95%-105%. | | | | |

# Range

The linearity was demonstrated from a nominal concentration range of approximately 5% to 120%. The accuracy was demonstrated from a nominal concentration range of approximately 5% to 150%. Hence, the demonstrated working range of the method (within both demonstrated linearity and accuracy range) is from a nominal concentration of 5% to 120%, which corresponds to a CX-4945 concentration from 11 µg/mL to 264 µg/mL.

# Precision

## Precision

A six-capsule dissolution profile was performed using the CX-4945 capsules (200 mg) as per the analytical test method (**Section 2**).

## Results and Discussion

All system suitability results were met.

The precision results are summarized in **Table 9-1**. All acceptance criteria were met.

**Figure 7** is a representative chromatogram of the sample solution.

Table 9-1. Precision results

(Notebook Reference: ARD-0270, pg. 40)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Vessel** | **% Dissolved** | | | | |
| **5 min** | **15 min** | **30 min** | **45 min** | **60 min** |
| 1 | 8.72 | 48.18 | 99.98 | 99.76 | 99.30 |
| 2 | 8.82 | 57.52 | 97.22 | 96.72 | 95.96 |
| 3 | 8.83 | 55.19 | 96.40 | 98.26 | 97.76 |
| 4 | 14.68 | 57.40 | 100.82 | 100.26 | 99.50 |
| 5 | 4.15 | 36.69 | 93.71 | 97.28 | 97.11 |
| 6 | 7.39 | 50.35 | 100.14 | 100.47 | 99.56 |
| **Mean** | **9** | **51** | **98** | **99** | **98** |
| **Min** | **4** | **37** | **94** | **97** | **96** |
| **Max** | **15** | **58** | **101** | **100** | **100** |
| **% RSD** | **38.9** | **15.6** | **2.8** | **1.6** | **1.5** |
| Acceptance Criteria:  • The % RSD for the dissolution results at 45 minutes is NMT 6% for mean results < 85% dissolved, and NMT 5% for mean results ≥ 85% dissolved. | | | | | |

# Filter Study

A filter study was performed to evaluate the suitability of the filters used (Whatman 0.45‑µm PVDF membrane filter) for the sample solution preparation.

## Filter Study on Dissolution Medium

A portion of the dissolution medium previously heated to about 37°C was filtered through a Whatman 0.45‑µm PVDF filter. The first 2 mL of the filtrate was collected and analyzed.

## Filter Study on Sample Solution

Filtered Sample:

A portion of the sample solution (prepared as per **Section 2.9**) was filtered through a Whatman 0.45‑µm PVDF filter. Aliquots were collected as showing in **Table 10-1**.

**Table 10-1. Collection of filtrate aliquots for filter study**

|  |  |  |
| --- | --- | --- |
| **Aliquot** | **Filtration Fraction (mL)** | **Volume Collected (mL)** |
| 1 | 0-3 | 3 |
| 2 | 3-6 | 3 |
| 3 | 6-9 | 3 |

Centrifuged Sample:

An aliquot of the sample solutions evaluated for the filter study was centrifuged at 10000 rpm (11400 RCF) for 10 minutes.

## Results and Discussion

All system suitability requirements were met.

There were no peaks attributed to the filter that were observed to affect the quantitation of the CX-4945 peak.

The results from the filter study on the sample solution are summarized in Table 9-2. All criteria were met for all evaluated aliquots.

Table 8-1. Precision results

(Notebook Reference: ARD-0307, pg. 23)

|  |  |  |  |
| --- | --- | --- | --- |
| **Aliquot** | **Filtration Fraction,  (mL)** | **Recovery,  (%)** | **Relative Recovery  (%)** |
| Centrifuge | — | 93.72 | — |
| 1 | 0-3 | 94.93 | 101 |
| 2 | 3-6 | 94.61 | 101 |
| 3 | 6-9 | 94.89 | 101 |
| Acceptance Criteria:  • The relative recovery of CX-4945 in each filtrate aliquot of the sample solution to the centrifuged sample solution is within 97 – 103%.. | | | |

# Stability Study

The standard and sample solutions was evaluated at normal laboratory environmental condition to determine the appropriate time frame for use. Their stabilities were determined by periodically evaluating the solutions for change in CX-4945 against freshly prepared.

Standard solution stability was considered from the time of preparation to the time of injection of the aged solution. Sample solution stability was considered from the time of initial injection to the time of injection of the aged solution.

## Procedure

The working standard prepared as per Section 2.8 was evaluated for stability at normal laboratory environmental conditions.

The dissolution sample solution (60 minute timepoint) was evaluated for stability at normal laboratory environmental conditions.

## Results and Discussion

The system suitability requirements were met at each evaluated interval. Each solution was injected once at each evaluation.

Working Standard Solution:

The working standard solution stability results are summarized in **Table 11-1**.

All criteria were met at each evaluated interval and condition.

The working standard solution was found to be stable for at least 5 days when stored at normal laboratory environmental conditions.

Table 11-1. Results from the stability study of the working standard solution

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Time** | **Condition** | **Recovery, (%)** | **Relative Recovery, (%)** | **Reference** |
| Initial | — | 100.00 | — | ARD-0270, pg. 43 |
| Day 2 | NLEC | 98.86 | 98.9 | ARD-0307, pg. 22 |
| Day 5 | NLEC | 99.84 | 99.8 | ARD-0247, pg. 95 |
| Acceptance Criteria:  • The standard solutions are considered stable if the relative recovery result at each time interval is within the range of 98.0 – 102.0%. | | | | |

Sample Solution:

The sample solution stability results are summarized in **Table 11-2**.

All criteria were met at each evaluated interval and condition.

The sample solution was found to be stable for at least 5 days when stored at normal laboratory environmental conditions.

Table 11-2. Results from the stability study of the sample solution

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Time** | **Condition** | **Recovery, (%)** | **Relative Recovery, (%)** | **Reference** |
| Initial | — | 101.14 | — | ARD-0307, pg. 24 |
| Day 2 | NLEC | 100.28 | 99 | ARD-0307, pg. 24 |
| Day 5 | NLEC | 101.17 | 100 | ARD-0247, pg. 95 |
| Acceptance Criteria:  • The sample solutions are considered stable if the relative recovery result at each time interval is within the range of 98 – 102%. | | | | |

# Conclusion

The method validation protocol PRO MV 0138-1 was successfully executed. The study findings are summarized as below. Based on the findings, the method is considered verified and suitable for intended use.

* Specificity (Interference): The Specificity was successfully demonstrated. There were no significantly interfering peaks found to elute at the retention time of the CX-4945 peak from the diluent interference, dissolution medium interference, and 2x placebo interference solutions.
* Linearity: The Linearity was demonstrated from a CX-4945 concentration of about 11 µg/mL to 264 µg/mL, which corresponds to 5% to 120% of the nominal concentration of the working standard solution.
* Accuracy: The Accuracy was demonstrated from a CX-4945 residual amount of about 11 mg/mL to 330 mg/mL, which corresponds to about 5% to 150% of the nominal sample solution concentration.
* Range: The demonstrated working range of the method (within both demonstrated linearity and accuracy range) is from a nominal concentration of 5% to 120%, which corresponds to a CX-4945 concentration from 11 µg/mL to 264 µg/mL.
* Precision: The Precision was successfully demonstrated.
* Filter: The Whatman 0.45‑µm PVDF membrane filters were demonstrated to be suitable for use in the sample solution preparation with the discard volume of the first 3 mL of the filtrate.
* Stability of the Standard Solution: The working standard solution was found to be stable for at least 5 days when stored at normal laboratory environmental conditions.
* Stability of the Sample Solution: The sample solution was found to be stable for at least 5 days when stored at normal laboratory environmental condition.

# Figures

Figure 1. A representative chromatogram of the working standard solution

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Figure 2. A representative chromatogram of the diluent interference solution



Figure 3. A representative chromatogram of the dissolution medium interference solution

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Figure 4. A representative chromatogram of the placebo interference solution



**Figure 5. The plot of the area response vs. concentration**

Figure 6. A Plot of Relative Response Factor vs. Concentration

Figure 7. A representative chromatogram of the sample solution

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# Changes/Deviations and Investigations

## Changes/Deviations from Protocol

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| **Protocol Section No.** | **Change/Deviation** |
| 2.1 | Sinker information was omitted. |
| 5 | The recovery study was initially performed as per stated procedure in the method verification protocol. The recovery at the 50% level was found not to meet the acceptance criteria. A review of the sample preparation found that the quantity of capsule was a 4.5x greater than what would typically be found. Hence, the sample preparation for the Accuracy study was modified so that the matrix would more adequately reflect real working conditions. The revised procedure is updated in this report (Section 6). |
| Table 8-1 | For each of the aliquots, the volume collected for each of the aliquots was incorrectly listed as 2 mL instead of 3 mL. |

## Investigations

There were no investigations.