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| **Protocol Reference** | PRO MV 0137-1, Effective: May. 11, 2021 |
| **Notebook References** | |  |  | | --- | --- | | **Notebooks** | **Pages** | | ARD-0308  ARD-0307  ARD-0309 | 1-50  1-6, 18-21  1-20 | |
| **Analysts** | Ran Li  Marjorie Cordero  Hinal Patel  Nadia Skoropad  Lin Sun |

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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 the execution of method verification protocol PRO MV 0137-1, which pertains to the verification of the *Assay (Content Uniformity* and *Blend Uniformity)*, *Related Substances* and *Identification by Retention Time* analytical procedure for CX-4945 (sodium salt) drug substance (Manufacturer: Carbogen Amcis AG) and CX-4945 capsules (200 mg) by Frontida BioPharm Analytical Research and Development (ARD) department.

Note that CX-4945 capsules contain the CX-4945 as a sodium salt. The label claim is calculated based on the free acid.

The method qualification of analytical procedure has been successfully performed by Alcami, the findings from which are summarized in corresponding method qualification report provided by Senhwa Biosciences, Inc., Report#: RPT 71442.00. The qualification of the method included and demonstrated the following method parameters/characteristics:

* System Suitability
* Specificity (Interference)
* Forced Degradation
* Linearity
* Accuracy by Recovery
* Method Repeatability
* Method Sensitivity (Reporting Limit)
* Filter Study
* Stability of Solutions

Appropriate verification studies were performed by the Frontida BioPharm ARD department in order to verify the suitability of the method and demonstrate the capability to perform the analysis.

The studies were performed in accordance with Frontida BioPharm’s Standard Operating Procedure (SOP) for Verification of Analytical Methods, SOP MPC QC/RD-017 (current version) and corresponding method verification protocol PRO MV 0137-1. The results were assessed as defined in the method verification protocol. Changes or deviations from the protocol are reflected in this report.

In order to verify the suitability of the test method and demonstrate the capability of Frontida BioPharm ARD department to perform this analysis, the following studies were performed:

* System Suitability
* Specificity (Interference and Identification)
* Forced Degradation[[1]](#footnote-1) (Oxidation by Peroxide and Metal Oxidation)
* Quantitation Limit
* Accuracy by Spiked Recovery
* Precision (Repeatability)
* Filtration Study
* Stability of Solutions

# Analytical Procedure

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

## Chromatographic Parameters

Table 2-1. HPLC Parameters

|  |  |
| --- | --- |
| **Column** | Phenomenex Gemini C18, 110 Å 100 mm x 4.6 mm, 3 µm  Part number: 00D-4439-E0 |
| **Mobile Phase A** | 0.1% TFA in Purified Water |
| **Mobile Phase B** | 0.05% TFA in Acetonitrile |
| **Needle Wash** | 50:50 Acetonitrile: Purified Water |
| **Needle Wash Setting** | Extended |
| **Gradient Program** | |  |  |  | | --- | --- | --- | | Time (min) | A (%) | B (%) | | 0 | 90 | 10 | | 2.0 | 50 | 50 | | 6.0 | 35 | 65 | | 8.0 | 90 | 10 | | 12.0 | 90 | 10 | |
| **Detection** | 227 nm |
| **Flow Rate** | 1.2 mL/min |
| **Column Temperature** | 30°C ± 5°C |
| **Injection Volume** | 10 μL |
| **Sampling Rate** | 10 points/sec |
| **Run Time** | 12 minutes |

## Reagents and Materials

* Purified Water, Millipore
* Acetonitrile, HPLC Grade
* Trifluoroacetic Acid (TFA), HPLC Grade
* CX-4945 (free acid) Standard, client provided
* CX-4945 Capsules composite placebo
* CX-4945 Capsules, 200 mg
* Millipore 0.45‑μm PVDF membrane filter

## 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.*

### Stock Standard Solution Preparation

Accurately weigh the equivalent of approximately 50 mg of CX-4945 free acid standard by quantitatively transferring into a 50-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 if necessary to dissolve. Allow solution to cool to room temperature, then dilute to volume with diluent and mix well.

The concentration of CX-4945 free acid is 1.0 mg/mL.

Prepare a check standard solution in a similar manner.

### Working Standard Solution Preparation

Dilute 5.0 mL of the stock standard solution to 50 mL with the Diluent. Mix well.

The concentration of CX-4945 free acid is 0.1 mg/mL.

Prepare a check standard solution in a similar manner.

## Intermediate Sensitivity Solution

Dilute 2.0 mL of the working standard solution to 100 mL with the Diluent. Mix well.

The concentration of CX-4945 free acid is 2.0 µg/mL (2.0% w/w%).

## Sensitivity Solution

Dilute 2.5 mL of the intermediate sensitivity solution to 100 mL with the Diluent. Mix well.

The concentration of CX-4945 free acid is 0.05 µg /mL (0.05% w/w%).

## Drug Substance Assay/RS Sample Solution Preparation

### Stock Sample Solution Preparation:

Accurately weigh and quantitatively transfer corrected amount of sample equivalent to 50 mg of CX-4945 in the free acid form (approximately 60 mg of CX-4945 as sodium salt) into a 50‑mL volumetric flask. Add about ¾ volume of diluent and mix to dissolve. Sonicate until completely dissolved. Allow solution to cool to room temperature, then dilute to volume with diluent and mix well.

### Working Sample Solution Preparation:

Dilute 5.0 mL of the stock sample solution to 50 mL with the diluent. Mix well.

The concentration of CX-4945 free acid is about 0.1 mg/mL.

## Drug Product Assay/RS Sample Solution Preparation

### Stock Sample Solution Preparation:

Accurately weigh 10 capsules and transferred the capsule contents into a single container and mix well. Weigh the empty capsule shells to determine an average capsule content.

Accurately weigh and transfer the equivalent of 2 capsules into a 200-mL volumetric flask. Add about ¾ volume of diluent and swirl to avoid clumping. Sonicate for 15 minutes with occasional swirling. Mechanically shake for 15 minutes. Allow solution to cool to room temperature, then dilute to volume with diluent and mix well. Filter an aliquot of the solution through a Millipore 0.45‑μm PVDF membrane filter, discarding the first 3 mL to waste.

### Working Sample Solution Preparation:

Dilute 5.0 mL of the stock sample solution to 100 mL with the diluent. Mix well.

The concentration of CX-4945 free acid is about 0.1 mg/mL.

## Drug Product CU Sample Solution Preparation

### Stock Sample Solution Preparation:

Accurately weigh 1 capsule and transfer entire contents into a 100-mL volumetric flask. Rinse capsule shells with diluent into the volumetric flask. Add about ¾ volume of diluent and swirl to avoid clumping. Sonicate for 15 minutes with occasional swirling. Mechanically shake for 15 minutes. Allow solution to cool to room temperature, then dilute to volume with diluent and mix well. Filter an aliquot of the solution through a Millipore 0.45‑μm PVDF membrane filter, discarding the first 3 mL to waste.

### Working Sample Solution Preparation:

Dilute 5.0 mL of the stock sample solution to 100 mL with the diluent. Mix well.

The concentration of CX-4945 free acid is about 0.1 mg/mL.

## Drug Product BU Sample Solution Preparation

### Stock Sample Solution Preparation:

Determine appropriate size of volumetric flask needed to prepare a sample solution in the range of 1.0 – 3.0 mg/mL CX-4945 free acid.

Transfer entire contents into an appropriate volumetric flask. Rinse bottle with diluent to effect complete transfer. Add about ¾ volume of diluent and swirl to avoid clumping. Sonicate for 15 minutes with occasional swirling. Mechanically shake for 15 minutes. Allow solution to cool to room temperature, then dilute to volume with diluent and mix well. Filter an aliquot of the solution through a Millipore 0.45‑μm PVDF membrane filter, discarding the first 3 mL to waste.

Allow the bottles to dry and then record weight.

### Working Sample Solution Preparation:

Dilute 5.0 mL of the stock sample solution to 100 mL with the diluent. Mix well.

## Procedure

Separately inject equal volumes (10 µL) of the diluent, sensitivity, standard (n=5), and sample solutions) – refer to example injection sequence below. Record the chromatograms and measure the peak area responses of the CX-4945 peak.

**Example Injection Sequence**:

|  |  |
| --- | --- |
| **Solution** | **Number of Injections** |
| Diluent | ≥1 |
| Sensitivity | 1 |
| Working Standard | 5 |
| Working Check Standard | 2 |
| Working Standard as Procedural Control Standard (PCS) | 1 |
| Working Sample Solution (Assay, RS, CU, BU, ID) | 1 |
| Working Standard as Procedural Control Standard (PCS) | 1 |

## System Suitability Requirements

* The diluent injection should have no peaks which significantly interfere (NMT 0.2% 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 S/N of CX-4945 peak from the injection of sensitivity solution ≥ 10.
* The mean Tailing Factor (Tf) for the CX-4945 peak 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 1.5%.
* The % RSD of the CX-4945 *retention time* from the five (5) consecutive injections of working standard solution is NMT 2.0%.
* Standard check agreement should be between 98.5 – 101.5%.

Note—The S/N requirement does not apply when only testing Assay, BU, or CU.

## Calculations

Calculate as follows:

DRUG SUBSTANCE ASSAY (% w/w, free acid, as is):

DRUG SUBSTANCE ASSAY (% w/w, free acid, on anhydrous, solvent free basis):

DRUG PRODUCT ASSAY (%LC):

CONTENT UNIFORMITY (%LC):

Calculate the content uniformity acceptance value (AV) as per cUSP <905>.

Acceptance Value =

Where:

|  |  |
| --- | --- |
| : | Mean of individual contents |
| k: | 2.4 (for sample size of 10 units) or k = 2.0 (for sample size of 30 units) |
| s: | Standard deviation of individual contents |
| 1M: | Case,  If 98.5% 101.5%, then M =  If < 98.5% then M = 98.5%  If > 101.5% then M = 101.5% |

BLEND UNIFORMITY (%LC):

RELATED SUBSTANCES (% area):

RETENTION TIME DIFFERENCE (% difference):

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 CX-4945 free acid standard, in mg

Wspl : Weight of CX-4945 Sample powder, in mg

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

: Volume of Stock Sample solution, in mL

ACC : Average capsule content in mg, salt form/capsule

MWC :

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

Rimp : The area response of individual impurity peak in the sample solution

Rtotal : Sum of all peak area responses of all peaks in the sample solution greater than or equal to 0.05%, excluding peaks observed in the diluent or solvent front

RTstd : Retention Time average from bracketing standard.

RTspl : Retention Time from Sample

# INSTRUMENTS/EQUIPMENT AND REAGENTS/MATERIALS

## Instruments and Equipment:

* Waters LC e2695 with 2998 PDA Detector (Instrument: ARD LC90, Calibration Due: 04/22)
* Sartorius Analytical Balances (Balance: ARD AB13, Calibration Due: 08/21; Balance: ARD AB18, Calibration Due: 07/21)
* Phenomenex Gemini C18, 110 Å 100 mm x 4.6 mm, 3 µm, Part number: 00D-4439-E0, S/N:H20-325856

## Reagents and Materials (Used in Method Verification)

* Purified Water, Millipore, In-house
* Acetonitrile (ACN), Mfr.: OmniSolv, Lot# 60358, Exp. Date: 03/24, Storage: RT
* Trifluoroacetic acid (TFA), Mfr.: Sigma, Lot# MKCL3567, Exp. Date: 10/21, Storage: RT
* CX-4945 (free acid) 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, Storage: RT
* CX-4945 Capsules composite placebo, Lot# NB071:002, Storage: RT
* CX-4945 Capsules, 200 mg, Mfr.: Senhwa Biosciences, Lot# B180393, Storage: RT
* Empty hard gelatin capsules, Mfr.: Capsugel, Lot# RL00311, Storage: RT
* Millipore PVDF 0.45-µm membrane filter, Lot# R0CA97854

# System Suitability/System Precision

The System Suitability and System Precision of the test method was performed and demonstrated as part of establishing system suitability for the subsequent verification studies.

## Results and Discussion

The system suitability was successfully established as per requirements described in **Section 2.14**. The results are summarized in the **Table 4-1** and **Table 4-2**. The successful establishment of system suitability is considered fulfillment of this study. **Figure 1** is a representative chromatogram of the working standard solution.

Table 4-1. System suitability requirements

(Notebook Reference: ARD-0308, pg. 16)

|  |  |
| --- | --- |
| **Requirement** | **Results from Standard injection** |
| Interference from Diluent | No interference at the retention time of CX-4945 |
| S/N of CX-4945 peak in the sensitivity solution | 19\* |
| Mean Tailing Factor (Tf) | 1.0 |
| \* The result of S/N for QL-1 was taken as the result for sensitivity solution.  System Suitability Requirements:  • The diluent injection should have no peaks which significantly interfere (NMT 0.2% 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 S/N of CX-4945 peak from the injection of sensitivity solution ≥ 10.  • The mean Tailing Factor (Tf) for the CX-4945 peak from the five (5) consecutive injections of working standard solution is NMT 2.0. | |

Table 4-2. System precision

(Notebook Reference: ARD-0308, pg. 15, 50)

|  |  |  |
| --- | --- | --- |
| **Standard** | **Area Response** | **RT (min)** |
| 1 | 3027746.1474 | 3.9515 |
| 2 | 3031047.2836 | 3.9531 |
| 3 | 3039954.9126 | 3.9528 |
| 4 | 3030385.5847 | 3.9457 |
| 5 | 3039135.1115 | 3.9513 |
| **%RSD** | **0.2** | **0.1** |
| **% Check Standard Recovery** | **99.9** | — |
| System Suitability Requirements:  • The % RSD of the CX-4945 peak area responses from the five (5) consecutive injections of working standard solution is NMT 1.5%.  • The % RSD of the CX-4945 retention time from the five (5) consecutive injections of working standard solution is NMT 2.0%.  • Standard check agreement should be between 98.5 – 101.5%. | | |

# Specificity (Interference)

## Diluent Interference Solution Preparation

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

## Placebo Interference Solution Preparation

### Stock Placebo Interference Solution Preparation

About 140 mg of CX-4945 capsule composite placebo was accurately weighed and quantitatively transferred into a 100‑mL volumetric flask. The flask was filled with diluent to ¾ of flask volume and swirled to avoid clumping, sonicated for 15 minutes with occasional swirling, and mechanically shaken for 15 minutes. The solution was cooled to room temperature, then diluted to volume with diluent and mixed well. An aliquot of the solution was filtered through a 0.45‑μm Millipore PVDF membrane filter, discarding the first 3 mL to waste.

### Placebo Interference Solution Preparation

A volume of 5.0 mL of the stock placebo interference solution was transferred into a 100-mL volumetric flask, diluted to volume with diluent, and mixed well.

## Sample Interference Solution Preparation

### Drug Substance Interference Solution Preparation

The sample solution prepared for **Section 9.1** was used.

### Drug Product Interference Solution Preparation

The sample solution prepared for **Section 9.2** was used.

## Results and Discussion

All system suitability requirements were met.

There were no significantly interfering peaks (NMT 0.2% relative to the average peak area of the CX-4945 peak from the five replicate injections of working standard) present at the retention time of CX-4945 peak from injections of the diluent interference and placebo interference solutions.

The resolution between CX-4945 and the closest eluting peak ≥ 0.05% were 10.1 and 10.2 for drug substance interference and drug product interference solutions, respectively.

All criteria were met.

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

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

**Figure 4** is a representative chromatogram of the drug substance interference solution.

**Figure 5** is a representative chromatogram of the drug product interference solution.

# Forced Degradation

Forced Degradation (FD) studies were performed on the composite placebo and drug product. The placebo and drug product were exposed to peroxide (~5% H2O2) and metal oxidative (50 mM FeCl3) conditions. The forced degradation was performed at the nominal working sample solution concentration of 0.1 mg/mL.

## Control Sample Solution Preparation

### Control Placebo Preparation

About 20 mg of composite placebo powder was accurately weighed and quantitatively transferred into a 250‑mL volumetric flask. A volume of 12.5 mL of purified water was transferred and gently swirled. The flask was filled with diluent to ¾ of flask volume and swirled to avoid clumping, sonicated for 15 minutes with occasional swirling, and mechanically shaken for 15 minutes. The solution was cooled to room temperature, then diluted to volume with diluent and mixed well. An aliquot of the control placebo solution was centrifuged at 10000 rpm (11400 RCF) for 10 minutes.

### Control Sample Preparation

About 43 mg of capsule content powder was accurately weighed and quantitatively transferred into a 250‑mL volumetric flask. A volume of 12.5 mL of purified water was transferred and gently swirled. The flask was filled with diluent to ¾ of flask volume and swirled to avoid clumping, sonicated for 15 minutes with occasional swirling, and mechanically shaken for 15 minutes. The solution was cooled to room temperature, then diluted to volume with diluent and mixed well. An aliquot of the control sample solution was centrifuged at 10000 rpm (11400 RCF) for 10 minutes.

## Oxidation by Peroxide (5% Hydrogen Peroxide)

### Peroxide Oxidation Blank Preparation

5% hydrogen peroxide solution:

A volume of 16.5 mL of the concentrated hydrogen peroxide was transferred into a 100-mL volumetric flask, diluted to volume with purified water, and mixed well.

A volume of 5.0 mL of the 5% hydrogen peroxide solution was transferred into a 100-mL volumetric flask, diluted to volume with diluent, and mixed well.

### Peroxide Oxidation Placebo Preparation

About 20 mg of composite placebo powder was accurately weighed and quantitatively transferred into a 250‑mL volumetric flask. A volume of 12.5 mL of 5% hydrogen peroxide solution was transferred and gently swirled. The flask was kept for 24 hours at ambient condition. The flask was filled with diluent to ¾ of flask volume and swirled to avoid clumping, sonicated for 15 minutes with occasional swirling, and mechanically shaken for 15 minutes. The solution was cooled to room temperature, then diluted to volume with diluent and mixed well. An aliquot of the peroxide oxidation placebo solution was centrifuged at 10000 rpm (11400 RCF) for 10 minutes.

### Peroxide Oxidation Sample Preparation

About 43 mg of capsule content powder was accurately weighed and quantitatively transferred into a 250‑mL volumetric flask. A volume of 12.5 mL of 5% hydrogen peroxide solution was transferred and gently swirled. The flask was kept for 24 hours at ambient condition. The flask was filled with diluent to ¾ of flask volume and swirled to avoid clumping, sonicated for 15 minutes with occasional swirling, and mechanically shaken for 15 minutes. The solution was cooled to room temperature, then diluted to volume with diluent and mixed well. An aliquot of the peroxide oxidation sample solution was centrifuged at 10000 rpm (11400 RCF) for 10 minutes.

## Metal Oxidation (50 mM Ferric Chloride)

### Metal Oxidation Blank Preparation

50 mM Ferric Chloride solution:

About 0.8 g of the ferric chloride (FeCl3) was accurately weighed and quantitatively transferred into a 100-mL volumetric flask, diluted to volume with purified water, and mixed well.

A volume of 5.0 mL of the 50 mM ferric chloride solution was transferred into a 100-mL volumetric flask, diluted to volume with diluent, and mixed well.

### Metal Oxidation Placebo Preparation

About 20 mg of composite placebo powder was accurately weighed and quantitatively transferred into a 250‑mL volumetric flask. A volume of 12.5 mL of 50 mM ferric chloride solution was transferred and gently swirled. The flask was kept for 6 days at ambient condition. The flask was filled with diluent to ¾ of flask volume and swirled to avoid clumping, sonicated for 15 minutes with occasional swirling, and mechanically shaken for 15 minutes. The solution was cooled to room temperature, then diluted to volume with diluent and mixed well. An aliquot of the metal oxidation placebo solution was centrifuged at 10000 rpm (11400 RCF) for 10 minutes.

### Metal Oxidation Sample Preparation

About 43 mg of capsule content powder was accurately weighed and quantitatively transferred into a 250‑mL volumetric flask. A volume of 12.5 mL of 50 mM ferric chloride solution was transferred and gently swirled. The flask was kept for 6 days at ambient condition. The flask was filled with diluent to ¾ of flask volume and swirled to avoid clumping, sonicated for 15 minutes with occasional swirling, and mechanically shaken for 15 minutes. The solution was cooled to room temperature, then diluted to volume with diluent and mixed well. An aliquot of the metal oxidation sample solution was centrifuged at 10000 rpm (11400 RCF) for 10 minutes.

## Results and Discussion

All system suitability requirements were met.

The forced degradation results are summarized in **Table 6-1** and **Table 6-2**. All criteria were met.

The CX-4945 drug product was found not be susceptible to degradation at the metal oxidation condition. There was no significant degradation of CX-4945 drug product at the metal oxidation condition after 6 days at ambient conditions.

The CX-4945 drug product was found to be susceptible to degradation at the peroxide oxidation condition. There was significant degradation of CX-4945 drug product (13%) when exposed to the peroxide oxidation condition for approximately 24 hours.

For both evaluated degradation conditions, the purity thresholds were greater than their respective purity angles, suggesting the absence of any degradation peaks, placebo or otherwise related to CX-4945, to significantly interfere with the CX-4945 peak.

Table 6-1: Forced Degradation Results of Peroxide Oxidation Sample

(Notebook Reference: ARD-0309, pg. 17)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Item** | **Recovery Against Control (%)** | **Resolution** | **Purity Threshold** | **Purity Angle** |
| Degradant 1 | 3.7018 | — | — | — |
| Degradant 2 | 3.6447 | 1.6 | — | — |
| Degradant 3 | 0.4211 | 11.7 | — | — |
| Diluent 1 | — | 5.3 | — | — |
| Degradant 4 | 0.1153 | 2.7 | — | — |
| Degradant 5 | 5.2451 | 2.1 | — | — |
| CX-4945 | 86.1890 | 5.4 | 2.6006 | 0.3132 |
| Blank/Placebo | — | 2.2 | — | — |
| Diluent 2 | — | 16.5 | — | — |
| Diluent 3 | — | 5.6 | — | — |
| **Total Degradation** | | 13.1281 | | |
| Acceptance Criteria:  • Degradation should be between 5% - 25%.  • The resolution between the active and the closest-eluting peak (if present at a level of ≥ 0.05%) is NLT 1.5.  • The resolution between any known impurity and the closest-eluting peak (if present at a level of ≥ 0.05%) is NLT 1.2.  • Degradation peaks ≥ 0.05% must be resolved from each other to the extent that all impurity peaks can be accurately quantified.  • Peak purity analysis of active peak from treated solutions indicate that the peak elutes as a spectrally homogenous peak (purity threshold > purity angle). | | | | |

Table 6-2: Forced Degradation Results of Metal Oxidation Sample

(Notebook Reference: ARD-0309, pg. 18)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Item** | **Recovery Against Control (%)** | **Resolution** | **Purity Threshold** | **Purity Angle** |
| Degradant 2 | 0.0928 | — | — | — |
| Diluent 1 | — | 18.3 | — | — |
| CX-4945 | 98.3412 | — | 4.3675 | 0.3866 |
| Blank/Placebo | — | 2.1 | — | — |
| Diluent 2 | — | — | — | — |
| **Total Degradation** | | 0.0928 | | |
| Acceptance Criteria:  • Degradation should be between 5% - 25%.  • The resolution between the active and the closest-eluting peak (if present at a level of ≥ 0.05%) is NLT 1.5.  • The resolution between any known impurity and the closest-eluting peak (if present at a level of ≥ 0.05%) is NLT 1.2.  • Degradation peaks ≥ 0.05% must be resolved from each other to the extent that all impurity peaks can be accurately quantified.  • Peak purity analysis of active peak from treated solutions indicate that the peak elutes as a spectrally homogenous peak (purity threshold > purity angle). | | | | |

**Figure 6** is the UV spectra of active and degradation products obtained for peroxide oxidation sample.

**Figure 7** is the UV spectra of active and degradation products obtained for metal oxidation sample.

**Figure 8** is the chromatogram of control sample solution.

**Figure 9** is the chromatogram of peroxide oxidation sample solution.

**Figure 10** is the chromatogram of metal oxidation sample solution.

# Quantitation Limit

The Quantitation Limit (QL) were evaluated at a concentration corresponding to an impurity level of 0.05%. The QL was represented by the sensitivity solution (**Section 2.8**). The signal-to-noise ratios (S/N) of CX-4945 peak were assessed in order to ensure that adequate sensitivity can be achieved at this level.

## Results and Discussion

All system suitability requirements were met.

The signal-to-noise ratios (S/N) of CX-4945 peak in the sensitivity solution are reported in **Table 7-1.** All criteria were met. **Figure 11** is a chromatogram of the QL solution (sensitivity solution).

The QL of the method was demonstrated at an impurity level of 0.05%, which corresponds to an impurity concentration of 0.05 µg /mL

Table 7-1: S/N of CX-4945 peak in the sensitivity solution.

(Notebook Reference: ARD-0308, pg. 16)

|  |  |  |  |
| --- | --- | --- | --- |
| **Item** | **Injection #** | **Area Response** | **S/N** |
| Sensitivity solution (QL) | 1 | 1564.9050 | 19 |
| 2 | 1630.1049 | 28 |
| 3 | 1630.4649 | 14 |
| 4 | 1609.7801 | 21 |
| 5 | 1594.0500 | 16 |
| 6 | 1566.9748 | 11 |
| **Mean** | | 1599.3799 | — |
| **RSD (%)** | | 2 | — |
| Acceptance Criteria:  • The S/N is NLT 10 in each injection.  • The % RSD of peak area responses is NMT 15% for the active. | | | |

# Accuracy by spiked Recovery

The accuracy of the method was assessed for the quantitation of the CX-4945 in the drug product.

The accuracy study was performed by spiking known amounts of CX-4945 drug substance onto a corresponding amount of CX-4945 capsule composite placebo.

For *Assay*, the accuracy was evaluated from CX-4945 concentrations corresponding to 50% to 150% of the nominal sample concentration of 0.1 mg/mL.

For *Impurities*, the accuracy were evaluated from concentrations corresponding to an impurity level of 0.1% to 0.3% of the nominal sample concentration of 0.1 mg/mL.

This study was performed using the CX-4945 sodium salt drug substance material. A control solution, prepared directly using the CX-4945 sodium salt drug substance, was used to evaluate the recovery in order to exclude the effects of any potency differences from using the drug substance material.

## Accuracy for Assay

### Recovery Sample Preparations

The CX-4945 capsule composite placebo and CX-4945 sodium salt drug substance was accurately weighed as directed in **Table 8-1** and quantitatively transferred into a 100‑mL volumetric flask. The flask was filled with diluent to ¾ of flask volume and swirled to avoid clumping, sonicated for 15 minutes with occasional swirling, and mechanically shaken for 15 minutes. The solution was cooled to room temperature, then diluted to volume with diluent and mixed well. An aliquot of the solution was filtered through a 0.45‑μm Millipore PVDF membrane filter, discarding the first 3 mL to waste.

A volume of 5.0 mL of the filtrate solution was transferred into a 100-mL volumetric flask, diluted to volume with diluent, and mixed well.

For each level, triplicate sample solution preparations were made.

**Table 8-1. Preparation of recovery sample solutions for assay**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Recovery Level** | **Nominal Concentration (%)** | **Weight of CX-4945 sodium salt (mg)** | **Weight of Placebo  (mg)** | **Flask Volume (mL)** | **Dilution** | **Approximate Concentration of CX‑4945  (mg/mL)** |
| R1 | 50% | 125 | 140 | 100 | 5.0 mL to 100 mL | 0.05 |
| R2 | 100% | 250 | 140 | 100 | 0.1 |
| R3 | 150% | 375 | 140 | 100 | 0.15 |

### Control/Reference Solution Preparation

The control/reference solution was prepared as per **Section 2.9** using the CX-4945 sodium salt drug substance.

## Accuracy for Impurities

### Spiking Solution Preparation

About 123 mg of CX-4945 sodium salt drug substance (equivalent to approximately 100 mg of CX-4945 as free acid) was accurately weighed and quantitatively transferred into a 100‑mL volumetric flask. The flask was filled with diluent to ¾ of flask volume and mixed to dissolve, sonicated until completely dissolved. The solution was cooled to room temperature, then diluted to volume with diluent and mixed well.

A volume of 5.0 mL of the above solution was transferred into a 100-mL volumetric flask, diluted to volume with diluent, and mixed well.

### Recovery Sample Preparations

About 140 mg of CX-4945 capsule composite placebo was accurately weighed and quantitatively transferred into a 100‑mL volumetric flask. The spiking solution was transferred as directed in **Table 8-2**. The flask was filled with diluent to ¾ of flask volume and swirled to avoid clumping, sonicated for 15 minutes with occasional swirling, and mechanically shaken for 15 minutes. The solution was cooled to room temperature, then diluted to volume with diluent and mixed well. An aliquot of the solution was filtered through a 0.45‑μm Millipore PVDF membrane filter, discarding the first 3 mL to waste.

A volume of 5.0 mL of the filtrate solution was transferred into a 100-mL volumetric flask, diluted to volume with diluent, and mixed well.

For each level, triplicate sample solution preparations were made.

**Table 8-2. Preparation of recovery sample solutions for impurities**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Recovery Level** | **Impurity Level  (%)** | **Volume of Spiking Solution (mL)** | **Weight of Placebo  (mg)** | **Flask Volume (mL)** | **Dilution** | **Approximate Concentration of CX‑4945  (µg/mL)** |
| R1 (QL) | 0.1 | 4.0 | 140 | 100 | 5.0 mL to 100 mL | 0.10 |
| R2 | 0.15 | 6.0 | 140 | 100 | 0.15 |
| R3 | 0.3 | 12.0 | 140 | 100 | 0.30 |

### Control/Reference Solution Preparation

A volume of 6.0 mL of the spiking solution (**Section 8.3.1**) was transferred into a 100-mL volumetric flask, diluted to volume with diluent, and mixed well.

A volume of 5.0 mL of the above solution was transferred into a 100-mL volumetric flask, diluted to volume with diluent, and mixed well.

## Results and Discussion

All system suitability requirements were met.

The recovery results for assay are summarized in **Table 8-3**. The recovery results for impurities are summarized in **Table 8-4.** All criteria were met.

Accuracy for assay of the method was demonstrated from about 0.05 mg/mL to 0.16 mg/mL, which corresponds to 54% to 161% of the specification.

Accuracy for impurities of the method was demonstrated from 0.11 mg/mL to 0.32 mg/mL, which corresponds to 0.1% to 0.3% of the specification (or 67% to 200% of the impurity specification).

Table 8-3. Recovery results for assay

(Notebook Reference: ARD-0308, pg. 3, 22, 36)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Recovery Level** | **Weight of CX-4945 Sodium Salt (mg)** | **Recovery (%)** | **Mean Recovery (%)** | **RSD (%)** |
| R1 (50%) | 125.6 | 100.9358 | 101.1 | 0.2 |
| 125.3 | 101.1036 |
| 125.1 | 101.3063 |
| R2 (100%) | 250.7 | 100.1066 | 100.4 | 0.3 |
| 250.8 | 100.5001 |
| 250.9 | 100.6513 |
| R3 (150%) | 376.6 | 98.8708 | 99.4 | 0.5 |
| 375.0 | 99.5276 |
| 375.7 | 99.7908 |
| Acceptance Criteria:  • The % RSD of the triplicate preparations within the same level is NMT 3.0%.  • The mean % recovery within the same level is between 98.0 – 102.0%. | | | | |

Table 8-4. Recovery results for impurities

(Notebook Reference: ARD-0308, pg. 5, 20-21)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Recovery Level** | **Weight of CX-4945 used for Spiking Solution  (mg)** | **Recovery (%)** | **Mean Recovery (%)** | **RSD (%)** |
| R1 (0.1%) | 123.2 | 91.8109 | 90 | 1.5 |
| 89.8700 |
| 89.1743 |
| R2 (0.15%) | 98.6649 | 93 | 5.0 |
| 90.2241 |
| 90.9030 |
| R3 (0.3%) | 89.9057 | 88 | 1.8 |
| 87.6898 |
| 86.8219 |
| Acceptance Criteria:  • The % RSD of the triplicate preparations within the same level is NMT 11.0%.  • The mean % recovery within the same level is between 80 – 120%. | | | | |

# Precision

## Drug Substance: Assay

Six (6) sample solutions were prepared using the CX-4945 drug substance as described in **Section 2.9**.

## Drug Product: Assay

Six (6) sample solutions were prepared using CX-4945 drug product capsules as described in **Section 2.10**.

## Drug Product: Content Uniformity

Ten (10) sample solutions were prepared as described in **Section 2.11**.

## Drug Product: Related Substances

Six (6) sample solutions spiked at the R2-0.15% level were prepared as described in **Section 8.2.2**.

## Results and Discussion

All system suitability requirements were met.

## For Drug Substance – Assay:

The precision results from the assay of drug substance are summarized in **Table 9-1**. All criteria were met.

Table 9-1: Precision results from the assay of drug substance.

(Notebook Reference: ARD-0308, pg. 17-18)

|  |  |  |
| --- | --- | --- |
| **Sample #** | **Assay1 (%)** | **Retention Time  (min)** |
| 1 | 101.8955 | 3.9550 |
| 2 | 101.8833 | 3.9540 |
| 3 | 101.7890 | 3.9523 |
| 4 | 101.8467 | 3.9560 |
| 5 | 101.6940 | 3.9567 |
| 6 | 101.7382 | 3.9521 |
| **Mean** | **101.8** | **3.9543** |
| **RSD (%)** | **0.1** | **—** |
| 1 Corrected for water content only.  Acceptance Criteria:  • The % RSD of the results from the Precision study (n=6) is NMT 3.0%.  • The average retention time of CX-4945 in the sample solution corresponds to that of the standard solution is within ± 2.0% (3.8717 - 4.0298 min). | | |

## For Drug Product – Assay:

The precision results from the assay of drug product are summarized in **Table 9-2**. All criteria were met.

Table 9-2: Precision results from the assay of drug product.

(Notebook Reference: ARD-0308, pg. 19)

|  |  |  |
| --- | --- | --- |
| **Sample #** | **Label Claim (%)** | **Retention Time  (min)** |
| 1 | 101.0171 | 3.9541 |
| 2 | 101.1554 | 3.9608 |
| 3 | 101.1247 | 3.9509 |
| 4 | 101.3519 | 3.9531 |
| 5 | 101.4398 | 3.9457 |
| 6 | 100.8812 | 3.9465 |
| **Mean** | **101.2** | **3.9518** |
| **RSD (%)** | **0.2** | **—** |
| Acceptance Criteria:  • The % RSD of the results from the Precision study (n=6) is NMT 3.0%.  • The retention time of CX-4945 in each sample solution corresponds to that of the standard solution is within ± 2.0% (3.8717 - 4.0298 min). | | |

## For Drug Product – Content Uniformity:

The results from the content uniformity of drug product are summarized in **Table 9-3**. All criteria were met.

Table 9-3: Results from the content uniformity of drug product.

(Notebook Reference: ARD-0309, pg. 16)

|  |  |
| --- | --- |
| **Sample #** | **LC (%)** |
| CU 01 | 99.8088 |
| CU 02 | 100.4806 |
| CU 03 | 98.4783 |
| CU 04 | 99.0898 |
| CU 05 | 97.3444 |
| CU 06 | 98.6392 |
| CU 07 | 98.4118 |
| CU 08 | 98.5656 |
| CU 09 | 97.9789 |
| CU 10 | 97.3307 |
| **Mean** | **98.6** |
| **STD Dev (%)** | **0.9923** |
| **AV** | **2.4** |
| Acceptance Criteria:  • The AV as calculated according to USP <905> is NMT 15.0. | |

## For Drug Product – Related Substances:

The results from the related substances of drug product are summarized in **Table 9-4**. All criteria were met.

Table 9-4: Results from the related substances of drug product.

(Notebook Reference: ARD-0308, pg. 21)

|  |  |  |
| --- | --- | --- |
| **Sample #** | **Impurity (%)** | **Absolute Difference (against mean)** |
| 1 | 0.1773 | 0.014 |
| 2 | 0.1621 | 0.001 |
| 3 | 0.1633 | 0.001 |
| 4 | 0.1576 | 0.005 |
| 5 | 0.1578 | 0.005 |
| 6 | 0.1588 | 0.004 |
| **Mean** | **0.1628** | — |
| **RSD (%)** | **4.6** | — |
| Acceptance Criteria:  • The % RSD of the impurity results ≥ 0.6% from the Precision study (n=6) is NMT 15.0%.  • The absolute difference between the individual and mean results for each impurity ≥ 0.05% and < 0.6 % must meet the criteria in **Table 9-5**.  **Table 9-5**. Absolute Difference Acceptance Criteria for Related substance   |  |  | | --- | --- | | % Related Substance | Absolute Difference | | ≥ 0.05 and ≤ 0.30 | NMT 0.10 | | > 0.30 and < 0.6 | NMT 0.20 | | | |

# Filter study

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

## Filter Study on Diluent

A portion of the diluent was filtered through the Millipore 0.45‑µm PVDF filter and the first 2 mL of filtrate was collected.

## Filter Study on Assay Sample Solution

Filtered Sample:

Portions of the assay stock sample solution for precision study in the **Section 9.1** was filtered through the Millipore 0.45‑µm PVDF filter, aliquots of the filtrate was collected as shown in **Table 10-1**. A volume of 2.5 mL of the filtrate solutions was transferred into a 50-mL volumetric flask, diluted to volume with diluent, and mixed well.

**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:

Additionally, a portion of the assay stock sample solution was centrifuged at 10000 rpm (11400 RCF) for 10 minutes to obtain a clear supernatant. A volume of 2.5 mL of the clear supernatant was transferred into the 50-mL volumetric flask, diluted to volume with diluent, and mixed well.

## Filter Study on Related Substance Sample Solution

Filtered Sample:

Portions of the stock recovery sample solution spiked at the R2-0.15% level (**Section 8.2.2)** was filtered through the Millipore 0.45‑µm PVDF filter, aliquots of the filtrate was collected as shown in **Table 10-1**. A volume of 2.5 mL of the filtrate solutions was transferred into a 50-mL volumetric flask, diluted to volume with diluent, and mixed well.

Centrifuged Sample:

Additionally, a portion of the sample solution was centrifuged at 10000 rpm (11400 RCF) for 10 minutes to obtain a clear supernatant. A volume of 2.5 mL of the clear supernatant was transferred into the 50-mL volumetric flask, diluted to volume with diluent, and mixed well.

## Results and Discussion

All system suitability requirements were met.

There were no peaks attributed to the filter that were observed to interfere with CX-4945. There was no significant adsorption of CX-4945 found at any of the discard volumes. For consistency, the discard volume of 3 mL will be maintained for the sample preparation.

The Millipore 0.45‑µm PVDF filters were found to be suitable for the sample preparation.

## Filter Study on Diluent:

There were no peaks attributed to the filter that were observed to interfere with CX-4945.

## Filter Study on Assay Sample Solution:

The results from the filter study on the assay sample solution for precision study are summarized in **Table 10-2**. All criteria were met.

Table 10-2. Results from filter study on sample solution - Assay

(Notebook Reference: ARD-0308, pg. 36-37)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Filter** | **Aliquot** | **Filtration Fraction (mL)** | **Recovery  (%)** | **Relative Recovery (%)** |
| Centrifuge | — | — | 100.9762 | — |
| Millipore 0.45 µm PVDF | 1 | 0-3 | 102.3294 | 101.3 |
| 2 | 3-6 | 101.8172 | 100.8 |
| 3 | 6-9 | 101.9401 | 101.0 |
| Acceptance Criteria:  • The relative recovery of CX-4945 in each filtrate aliquot of the sample solution to the centrifuged sample solution is within 98.0 – 102.0%. | | | | |

## Filter Study on Related Substance Sample Solution:

The results from the filter study on the related substance sample solution spiked at the R2-0.15% level are summarized in **Table 10-3**. All criteria were met.

Table 10-3. Results from filter study on sample solution - Related Substance

(Notebook Reference: ARD-0308, pg. 37-39)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Filter** | **Aliquot** | **Filtration Fraction (mL)** | **Recovery (%)** | **Relative Recovery (%)** | **Impurity (%)** | **Absolute Difference (against centrifuge)** |
| Centrifuge | — | — | 105.5815 | — | 0.1577 | — |
| Millipore 0.45 µm PVDF | 1 | 0-3 | 92.2123 | 87.3 | 0.1377 | 0.02 |
| 2 | 3-6 | 104.7834 | 99.2 | 0.1565 | 0.001 |
| 3 | 6-9 | 105.0776 | 99.5 | 0.1570 | 0.001 |
| Acceptance Criteria:  • For related substances ≥ 0.05% and < 0.4%, the absolute difference of the filtered sample result from the centrifuged sample result is NMT 0.10%.  • For related substances ≥ 0.4%, the percent recovery of CX-4945 in each filtrate aliquot of the sample solution to the centrifuged sample solution is within 85.0 – 115.0%. | | | | | | |

# Stability Study

The standard and sample solutions (drug substance and drug product) were evaluated at normal laboratory environmental condition (NLEC) 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 or qualified standard solutions.

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.

## 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.

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

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

|  |  |  |  |
| --- | --- | --- | --- |
| **Time** | **Recovery,  (%)** | **Relative Recovery, (%)** | **Reference** |
| Initial | 99.8659 | — | ARD-0308, pg. 15 |
| Day 2 | 100.1568 | 100.3 | ARD-0308, pg. 33 |
| Day 5 | 98.5780 | 98.7 | ARD-0308, pg. 44 |
| Acceptance Criteria:  • The standard solutions are considered stable if the relative recovery result at each time interval is within the range of 98.5 – 101.5%. | | | |

Sample Solution – Drug Substance:

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

The sample solution for drug substance was evaluated for 5 days. There was no change in impurities at each evaluation interval. All criteria were met at the day 2 evaluation. The relative recovery criterion was not met at the day 5 evaluation.

Therefore, the sample solution for drug substance will be considered stable for 2 days when stored at normal laboratory environmental condition.

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

|  |  |  |  |
| --- | --- | --- | --- |
| **Time** | **Recovery,  (%)** | **Relative Recovery, (%)** | **Reference** |
| Initial | 101.8955 | — | ARD-0308, pg. 17 |
| Day 2 | 101.4999 | 99.6 | ARD-0308, pg. 34 |
| Day 5 | 99.9391 | 98.1 | ARD-0308, pg. 45 |
| Acceptance Criteria:  • The sample solutions are considered stable if the relative recovery result at each time interval is within the range of 98.5 – 101.5%.  • For each related substance ≥ 0.05% and < 0.4%, the absolute difference of the aged sample result from the initial sample result is NMT 0.10%.  • For each related substance ≥ 0.4%, the relative % impurity in the aged sample solution to the initial sample solution is within 85.0 – 115.0%. | | | |

Sample Solution – Drug Product:

The sample solution for drug product stability results are summarized in **Table 11-3**.

The sample solution for drug substance was evaluated for 5 days. There was no change in impurities at each evaluation interval. All criteria were met at each evaluation interval.

The sample solution for drug product will be considered stable for 5 days when stored at normal laboratory environmental condition.

Table 11-3. Results from the stability study of the sample solution - Drug Product

|  |  |  |  |
| --- | --- | --- | --- |
| **Time** | **Recovery,  (%)** | **Relative Recovery, (%)** | **Reference** |
| Initial | 101.0171 | — | ARD-0308, pg. 19 |
| Day 2 | 100.6981 | 99.7 | ARD-0308, pg. 35 |
| Day 5 | 99.4775 | 98.5 | ARD-0308, pg. 45-46 |
| Acceptance Criteria:  • The sample solutions are considered stable if the relative recovery result at each time interval is within the range of 98.0 – 102.0%.  • For each related substance ≥ 0.05% and < 0.4%, the absolute difference of the aged sample result from the initial sample result is NMT 0.10%.  • For each related substance ≥ 0.4%, the relative % impurity in the aged sample solution to the initial sample solution is within 85.0 – 115.0%. | | | |

# Identification by Retention Time (RT)

Verification of the Identification by Retention Time were performed and demonstrated as part of establishing system suitability (**Section 2.14**) and execution of the Precision study for Assay (**Section** **9.1** and **9.2**). The successful establishment and completion of these studies are considered fulfillment of the *Identification by RT*.

## Results and Discussion

All system suitability requirements were met.

The retention time results from the system suitability are summarized in **Table 4-2**

The retention time results from the assay of drug substance and drug product are summarized in **Table 9-1** and **Table 9-2**. All criteria were met.

# Conclusion

The method verification protocol PRO MV 0137-1 was successfully executed. The study findings are summarized below. Based on the findings, the CX-4945 (Silmitasertib) Drug Substance and Capsules, 200 mg: *Assay*, *Related Substances*, *Content Uniformity*, *Blend Uniformity* and *Identification* *by Retention Time* method is considered verified and suitable for its intended use.

* Specificity (Interference): There were no significantly interfering peaks found to elute at the retention time of the CX-4945 peak from the diluent interference and placebo interference solutions. The resolution between CX-4945 and the closest eluting peak ≥ 0.05% were 10.1 and 10.2 for drug substance interference and drug product interference solutions, respectively.
* Forced Degradation: The CX-4945 drug product was found to be susceptible to degradation at the peroxide oxidation condition, but not susceptible to degradation at the metal oxidation conditions. For both evaluated degradation conditions, the purity thresholds were greater than their respective purity angles, suggesting the absence of any degradation peaks, placebo or otherwise related to CX-4945, to significantly interfere with the CX-4945 peak.
* Quantitation Limit (QL): The QL of the method was demonstrated at an impurity level of 0.05%, which corresponds to an impurity concentration of 0.05 µg/mL.
* Accuracy: Accuracy for *Assay*/*Blend* *Uniformity*/*Content* *Uniformity* was demonstrated from about 0.05 mg/mL to 0.16 mg/mL, which corresponds to 54% to 161% of the specification. Accuracy for *Related Substances* was demonstrated from about 0.11 µg/mL to 0.32 µg/mL, which corresponds to an impurity level of 0.1% to 0.3% of the specification (or 67% to 200% of the impurity specification).
* Precision: The precision of the method was successfully demonstrated for the *Assay*, *Related Substances*, and *Identification by RT* of drug substance and *Assay*, *Blend Uniformity*, *Content Uniformity*, *Related Substances*, and *Identification by RT* of drug product.
* Filter Study: The Millipore 0.45‑µm PVDF filter was demonstrated to be suitable for use in the sample solution preparation at the discard volume of 3 mL.
* 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 condition
* Stability of the Sample Solution – Drug Substance: The stability of the sample solution for drug substance was established as 2 days stored at normal laboratory environmental condition.
* Stability of the Sample Solution – Drug Product: The stability of the sample solution for drug product was established as 5 days stored at normal laboratory environmental condition.

# figures

**Figure 1.** A representative chromatogram of the working standard solution.



**Figure 2.** A representative chromatogram of the diluent interference solution.



**Figure 3.** A representative chromatogram of the placebo interference solution.



**Figure 4.** A representative chromatogram of the drug substance interference solution.



**Figure 5.** A representative chromatogram of the drug product interference solution.

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**Figure 6.** The UV spectra of active and degradation products obtained for peroxide oxidation sample.



**Figure 7**. The UV spectra of active and degradation products obtained for metal oxidation sample.



**Figure 8.** The chromatogram of control sample solution.

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**Figure 9.** The chromatogram of peroxide oxidation sample solution.



**Figure 10.** The chromatogram of metal oxidation sample solution.

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# Changes/deviations and Investigations

## Changes to and Deviations from the Protocol

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| --- | --- |
| **Protocol Section No.** | **Change/Deviation** |
| 2.15 | The note for the calculation of the drug substance assay is incorrect and not applicable. For correction of assay (anhydrous, solvent free basis), the water and residual solvents content should be determined within appropriate timeframe of assay evaluation. |
| 8.3 | During initial execution of *Precision* study for Content Uniformity, the acceptance criteria (AV NMT 15.0) was not met. Reviewing the results, there was one result that was significantly lower (75.74%) than the other nine results (range from 99.25% - 101.99%). Investigative confirmatory injections were made by reinjecting the original vial and from the original working solution and rediluting the stock solution. Based on the confirmatory injections, the root cause of the original result was determined to likely be due to inadequate mixing of the stock sample solution. The original study results were invalidated and the study was repeated. |
| 9.2 | The procedure for the preparation of the working sample after filtering and centrifuging was omitted. A dilution of 2.5 mL of filtrate/supernatant to 50 mL with diluent was made. |

## Investigations

None

1. Based on Alcami’s method validation report (Report#: RPT 71442.00), degradation of the drug product was produced only in oxidation by peroxide conditions. Hence, of the forced degradation conditions performed by Alcami, only the oxidation by peroxide condition of the drug product were performed herein as part of method verification. In addition, forced degradation of the drug product by metal oxidation, a condition not previously evaluated, were performed. [↑](#footnote-ref-1)