METHOD VALIDATION REPORT

CX-4945 (Silmitasertib) Tablets, 500 mg

ASSAY, RELATED SUBSTANCES, CONTENT UNIFORMITY, BLEND UNIFORMITY AND IDENTIFICATION BY RETENTION TIME METHOD BY HPLC

DOCUMENT #: RPT MV 0188-1

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Protocol Reference	Method Validation Protocol PRO MV-0176-2 (Effective date: 12/07/2021)
Notebook Reference	**Notebook** **Page** ARD-0377 30-35 ARD-0378 50-86 ARD-0379 62-136
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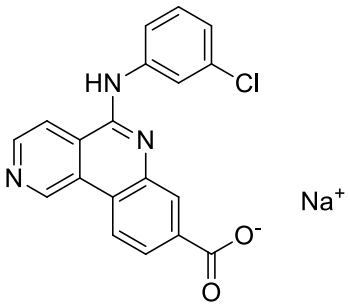
1 INTRODUCTION

CX-4945 Sodium Salt (Formula: $C_{19}H_{11}ClN_3O_2Na$; 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 the method validation protocol PRO MV 0176-2, which pertains to the validation of the *Assay (Content Uniformity* and *Blend Uniformity), Related Substances* and *Identification by Retention Time* analytical procedure for CX-4945 Tablets (500 mg).

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

The proposed formulation of CX-4945 Tablets, 500 mg is summarized in **Table 1-1**.

Table 1-1. Proposed formulation of CX-4945 Tablets, 500 mg

Ingredients	%w/w	mg/unit
Intra Granular		
CX-4945 (sodium salt) (a)	71.33	535.00
Mannogem EZ Spray Dried Mannitol	15.67	117.50
Hydroxy Propyl Cellulose (Klucel EF)	1.00	7.50
Croscarmellose Sodium, NF (Ac-Di-Sol)	5.00	37.50
Sodium Lauryl Sulfate(in solution)	1.00	7.50
Sodium Lauryl Sulfate	4.00	30.00
Purified Water	N/A	N/A
Granulation Total	**98.00**	**735.00**
Extra Granular		
Croscarmellose Sodium, NF (Ac-Di-Sol)	1.50	11.25
Magnesium Stearate, NF [Vegetable Source]	0.50	3.75
Fill Weight	**100.0**	**750.00**

Appropriate validation studies were performed by the Frontida BioPharm ARD department in order to demonstrate that the method is suitable for intended use.

The following studies were performed:

- System Suitability
- Specificity (Interference and Identification)
- Forced Degradation
- Linearity
- Quantitation Limit
- Accuracy by Spiked Recovery
- Precision
- Intermediate Precision
- Filtration Study
- Solution stability

The studies were performed in accordance with Frontida BioPharm Standard Operating Procedure (SOP) for Validation of Analytical Methods, SOP MPC QC/RD-017 (current version) based on ICH guidelines Q2 (R1).

2 ANALYTICAL PROCEDURE

2.1 Chromatographic Parameters

Table 2-1. HPLC Parameters

Column	Agilent Zorbax SB-C18 150 mm x 4.6 mm, 3.5 µm Part number: 863953-902
Mobile Phase A	0.1% TFA in Purified Water and Acetonitrile (90:10)
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 (%)** 0.00 / 100 / 0 0.25 / 100 / 0 3.50 / 55.5 / 44.5 6.50 / 40 / 60 8.50 / 100 / 0 12.5 / 100 / 0
Detection	227 nm
Flow Rate	1.2 mL/min
Column Temperature	30°C ± 3°C
Injection Volume	5 µL
Sampling Rate	10 points/sec
Run Time	12.5 minutes

2.2 Reagents and Materials

- Purified Water, Millipore
- Acetonitrile, HPLC Grade
- Trifluoroacetic Acid (TFA), HPLC Grade
- CX-4945 (free acid) Standard, client provided
- CX-4945 Tablets composite placebo
- CX-4945 Tablets, 500 mg
- Impurity C-028349
- Impurity C-028350
- Millipore 0.45-µm PVDF membrane filter

2.3 Mobile Phase A Preparation (0.1% TFA in water and Acetonitrile, 90:10)

Transfer 1.0 mL of TFA into a suitable flask containing 900 mL of purified water and 100 mL of acetonitrile. Mix well.

2.4 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.

2.5 Diluent Preparation

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

2.6 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.

2.6.1 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:

$\frac{50\ \text{mg}}{P}$, 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.

2.6.2 Working Standard Solution Preparation

Dilute 10.0 mL of the stock standard solution to 50 mL with the Diluent. Mix well.

The concentration of CX-4945 free acid is 0.2 mg/mL.

Prepare a check standard solution in a similar manner.

2.7 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 4.0 µg/mL (2.0% w/w%).

2.8 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 0.1 µg/mL (0.05% w/w%).

2.9 Placebo Solution Preparation

2.9.1 Stock Placebo Solution Preparation

Accurately weigh and quantitatively transfer about 250 mg of CX-4945 tablet composite placebo into a 250-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 0.45 µm Millipore PVDF membrane filter, discarding the first 3 mL to waste.

2.9.2 Working Placebo Solution Preparation

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

2.10 Assay and Related Substances Sample Solution Preparation

Determine the average tablet weight (ATW) of NLT 10 tablets.

$$\text{ATW} = \frac{Weight\ of\ NLT\ 10\ Tablets}{Quantity\ of\ NLT\ 10\ \ Tablets\ Weighed}$$

Grind tablets into a fine, uniform powder using a mortar and pestle.

Accurately weigh an amount of ground powder equivalent of 500 mg of CX-4945 as follows:

$$\text{Equivalent to (500 mg)} = \frac{500\ mg\ x\ ATW}{Label\ claim}$$

Transfer weight into a 250-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.

2.10.1 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 0.2 mg/mL.

2.11 Content Uniformity Sample Solution Preparation

2.11.1 Stock Sample Solution Preparation:

Accurately weigh 1 tablet and transfer into a 250-mL volumetric flask. Add about ¾ volume of diluent and swirl to avoid clumping. Mechanically shake for 15 minutes. Sonicate for 30 minutes with occasional swirling. Mechanically shake for an additional 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.

2.11.2 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 0.2 mg/mL.

2.12 Blend Uniformity Sample Solution Preparation

2.12.1 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.

2.12.2 Working Sample Solution Preparation:

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

2.13 Procedure

Separately inject equal volumes (5 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
Placebo Solution	1
Working Standard	5
Working Check Standard	1
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

2.14 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 (T_f) 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 2.0%.
- 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 98.0 – 102.0%.

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

2.15 Calculations

Calculate as follows:

DRUG PRODUCT ASSAY (%LC):

$$\%LC = \frac{R_{spl}}{R_s} \times \frac{W_s\ (mg) \times P}{50\ (mL)} \times \frac{10.0\ (mL)}{50\ (mL)} \times \frac{250\ (mL) \times ATW}{W_{spl}\ (mg)} \times \frac{50\ (mL)}{5.0\ (mL)} \times \frac{100\%}{LC}$$

CONTENT UNIFORMITY (%LC):

$$\%LC = \frac{R_{spl}}{R_s} \times \frac{W_s\ (mg) \times P}{50\ (mL)} \times \frac{10.0\ (mL)}{50\ (mL)} \times \frac{250\ (mL)}{1\ tablet} \times \frac{50\ (mL)}{5.0\ (mL)} \times \frac{100\%}{LC}$$

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

$$\text{Acceptance Value} = |M - \bar{X}| + ks$$

Where:

$\bar{X}$: 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
[1]M: Case,
If $98.5\% \leq \bar{X} \leq 101.5\%$, then $M = \bar{X}$
If $\bar{X} < 98.5\%$ then M = 98.5%
If $\bar{X} > 101.5\%$ then M = 101.5%

BLEND UNIFORMITY (%LC):

$$\%LC = \frac{R_{spl}}{R_s} \times \frac{W_s\ (mg) \times P}{50\ (mL)} \times \frac{10.0\ (mL)}{50\ (mL)} \times \frac{V_{spl}\ (mL)}{W_{spl}(mg)} \times \frac{50\ (mL)}{5.0\ (mL)} \times \frac{750\ mg}{LC} \times 100\%$$

RELATED SUBSTANCES (% area):

$$\%\ \text{Impurity} = \frac{R_{imp}}{R_{total}} \times 100\%$$

RETENTION TIME DIFFERENCE (% difference):

$$\%\ \text{Difference} = \frac{RT_{std} - RT_{spl}}{RT_{std}} \times 100\%$$

Where,

R_{spl}	:	The area response of CX-4945 in the sample solution
R_s	:	The area response of CX-4945 in the standard solution
Ws	:	Weight of CX-4945 free acid standard, in mg
W_{spl}	:	Weight of CX-4945 Sample, in mg
P	:	Purity of the CX-4945 free acid standard expressed as % Purity/100%

V_{spl} : Volume of Stock Sample solution, in mL

ATW : Average Tablet Weight in mg

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

R_{imp} : The area response of individual impurity peak in the sample solution

R_{total} : 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

RT_{std} : Retention Time average from bracketing standard, in min

RT_{spl} : Retention Time from Sample, in min

Note: The molecular weights of CX-4945 are as follow:

CX-4945 in free acid form: 349.77 g/mol

CX-4945 sodium salt form: 371.75 g/mol

3 INSTRUMENTS/EQUIPMENT AND REAGENTS/MATERIALS USED FOR VALIDATION

3.1 Instruments and Equipment

- Waters Alliance 2695 HPLC equipped with 2489 UV Detector
  - Instrument #: ARDLC80, Cal due: 08/22
- Waters Alliance 2695 HPLC equipped with 2998 PDA Detector
  - Instrument #: ARDLC90, Cal due: 04/22
- Chromatographic Column: Agilent Zorbax SB-C18, 150 mm x 4.6 mm, 3.5 µm, Part #: 863953-902
  - S/N: USEG024618
  - S/N: USEG025001

3.2 Reagents and Materials

- Purified Water, Millipore
- Acetonitrile, HPLC Grade, Mfr.: OmniSolv, Lot# 61308, Exp. Date: 10/24, Storage: RT
- Trifluoroacetic Acid (TFA), HPLC Grade, Mfr.: Alfa Aesar, Lot# R15H748, Exp. Date: 09/22, Storage: RT
- CX-4945 (free acid) Standard, client provided, Mfr.: Carbogen Amcis AG, Lot#: AA-023568-A-1-10 crude 2 # 1, Exp. Date: 09/22, Storage: Purity: 95.16%, Storage: RT

- CX-4945 sodium salt drug substance, Mfr.: Carbogen Amcis AG, Lot#: CA18-0842, Purity: 88.4%, Storage: RT
- CX-4945 Tablets composite placebo, Lot#: NB1766:39, Storage: RT
- CX-4945 Tablets, 500 mg, Lot#: NB1766:14 (3 months, Intermediate LT), Storage: RT
- CX-4945 Impurity 1, C-028349-SRS-01, Mfr.: Carbogen Amcis AG, Lot#: NE028349-A-1-4 crude# 2-1
- CX-4945 Impurity 2, C-028350-SRS-01, Mfr.: Carbogen Amcis AG, Lot#: NE028350-A-1-2 crude# 1-1
- Millipore 0.45-µm PVDF membrane filter, Lot#: R1HB94125

4 SYSTEM SUITABILITY

The System Suitability of the test method was performed and demonstrated as part of establishing system suitability for the subsequent validation studies. The successful establishment of the system suitability requirements (as described in **Section 2.14**) was considered fulfillment of this study.

4.1 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**.

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

Figure 1a is an expanded chromatogram of the working standard solution.

Table 4-1. System suitability requirements
(Notebook Reference: ARD-0379, pg. 90)

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	32
Mean Tailing Factor (T_f)	1.1
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 (T_f) 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-0379, pg. 86, 89, 90)

Standard	Area Response	RT (min)
1	3233046.3835	6.1825
2	3241270.1694	6.1733
3	3231976.7168	6.1852
4	3232325.9019	6.1701
5	3248725.0334	6.1778
%RSD	**0.2**	**0.1**
% Check Standard Recovery	**99.5**	

System Suitability Requirements:

• The % RSD of the CX-4945 peak area responses from the five (5) consecutive injections of working standard solution is NMT 2.0%.
• 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 98.0%-102.0%.

5 SPECIFICITY (INTERFERENCE)

5.1 Diluent Interference Solution Preparation

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

5.2 Placebo Interference Solution Preparation

A placebo solution was prepared as directed in **Section 2.9**.

5.3 Specificity (Impurity) Identification (ID) Solution Preparation

5.3.1 Stock Impurity ID Solutions Preparation:

About 15 mg each of Impurity C-028349 and Impurity C-028350 was accurately weighed and quantitatively transferred into separate 100-mL volumetric flasks. To each flask, diluent was added to fill about half the flask volume. The flasks were sonicated to dissolve the impurities. The solutions were allowed to cool to room temperature. Then, the flaks were diluted to volume with diluent and mixed well.

5.3.2 Intermediate Impurity ID Solution Preparation:

5.0 mL of each Stock Impurity ID solution were transferred into separate 250-mL volumetric flasks. The flasks were diluted to volume with diluent and mixed well.

5.3.3 Working Impurity ID Solution Preparation:

5.0 mL of each Intermediate Impurity ID solution were transferred into separate 50-mL volumetric flasks. The flaks were diluted to volume with diluent and mixed well.

The concentration of each impurity was about 0.3 µg/mL.

5.4 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 impurities did not show any significantly interfering peaks near the retention time of the CX-4945 peak.

The peaks from CX-4945 and the two impurities – Impurity C-028349 and Impurity C-028350 – were identified based on the standard solution and working impurity ID solutions, respectively. CX-4945 and its impurities were well separated from one another. The retention times and corresponding relative retention times of the specified impurities (to CX-4945) are summarized in **Table 5-1**.

All criteria were met.

Table 5-1. Retention times and relative retention times

(Notebook Ref: ARD-0379, Pg. 112)

Component	Retention Time (RT), minutes	Relative Retention Time
CX-4945 Impurity 1, C028349	5.2943	0.859
CX-4945 Impurity 1, C028350	3.8991	0.632
CX-4945	6.1626[1]	-

[1] Corresponds to mean of the procedural control standards

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

Figure 2a is an expanded 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 CX-4945 Impurity 1 working impurity ID solution.

Figure 5 is a representative chromatogram of the CX-4945 Impurity 2 working impurity ID solution.

6 FORCED DEGRADATION

Forced Degradation (FD) studies were performed on the composite placebo and drug product. The placebo and drug product were exposed to the following conditions: elevated temperature, ambient, UV photolysis, acid and base hydrolysis, and oxidation by peroxide (~5% H_2O_2).

6.1 Control Sample Solution Preparation

6.1.1 Control Placebo Preparation

About 40 mg of composite placebo powder was accurately weighed and quantitatively transferred into a 250-mL volumetric flask. 12.5 mL of purified water was added and the flask was gently swirled. The flask was filled with diluent to ¾ of flask volume and swirled to avoid clumping. The flask was sonicated for 15 minutes with occasional swirling. The flask was mechanically shaken for 15 minutes. The solution was allowed to cool 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.

6.1.2 Control Sample Preparation

About 86 mg of tablet powder was accurately weighed and quantitatively transferred into a 250-mL volumetric flask. 12.5 mL of purified water was added and the flask was gently swirl. The flask was filled with diluent to ¾ of flask volume and swirled to avoid clumping. The flask was sonicated for 15 minutes with occasional swirling. The flask was mechanically shaken for 15 minutes. The solution was allowed solution to cool 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.

6.2 Elevated Temperature Condition

Separate portions of the composite placebo powder (about 40 mg) and tablet powder (about 86 mg) were transferred into separate 250-mL volumetric flasks. The samples were placed in an oven at 105°C for a minimum of 3 hours.

Following the elapse of the minimum time, notes of any physical changes that occurred were taken. Sample solutions were prepared similarly as directed in **Section 6.1** except using the sample specimen exposed to elevated temperatures.

6.3 Short Wavelength UV Condition

Separate portions of composite placebo powder and tablet powder were transferred into suitable containers. Samples were placed under short wavelength UV light for at least 7 days.

Following the elapse of the minimum time, notes of any physical changes that occurred were taken. Sample solutions were prepared similarly as directed in **Section 6.1** except using the sample specimen exposed to the UV light.

6.4 Ambient Condition

Separate portions of composite placebo powder and tablet powder were transferred into suitable containers. Samples were placed under ambient conditions for at least 7 days.

Following the elapse of the minimum time, notes of any physical changes that occurred were taken. Sample solutions were prepared similarly as directed in **Section 6.1** except using the sample specimen exposed to ambient conditions.

6.5 Acid Hydrolysis Condition

6.5.1 Acid Blank Solution Preparation

Equal volumes, 5.0 mL, of 1 N hydrochloric acid and 1 N sodium hydroxide were transferred into a 100-mL volumetric flask. The flask was diluted to volume with diluent and mixed well.

6.5.2 Acid Hydrolysis Placebo Solution Preparation

About 40 mg of composite placebo powder was accurately weighed and quantitatively transferred into a 250-mL volumetric flask. 12.5 mL of 1 N Hydrochloric Acid was added and the flask was swirled. The solution was allowed to stand for 24 hours at ambient conditions. The solution was neutralized with 12.5 mL of 1 N Sodium Hydroxide solution. The flask was filled with diluent to ¾ of flask volume and swirled to avoid clumping. The flask was sonicated for 15 minutes with occasional swirling, and mechanically shaken for 15 minutes. The solution was allowed to cool to room temperature, then diluted to volume with diluent and mixed well. An aliquot of the placebo solution was centrifuged at 10000 rpm (11400 RCF) for 10 minutes.

6.5.3 Acid Hydrolysis Sample Solution Preparation

About 86 mg of tablet powder was accurately weighed and quantitatively transferred into a 250-mL volumetric flask. 12.5 mL of 1N Hydrochloric

Acid was added and the flask was swirled. The solution was allowed to stand for 24 hours at ambient conditions. The solution was neutralized with 12.5 mL of 1 N Sodium Hydroxide solution. The flask was filled with diluent to ¾ of flask volume and the flask was swirled to avoid clumping. The flask was sonicated for 15 minutes with occasional swirling and mechanically shaken for 15 minutes. The solution was allowed to cool to room temperature, then diluted to volume with diluent and mixed well. An aliquot of the sample solution was centrifuged at 10000 rpm (11400 RCF) for 10 minutes.

6.6 Base hydrolysis Condition

6.6.1 Base Blank Solution Preparation

Refer to **Section 6.5.1**.

6.6.2 Base Hydrolysis Placebo Solution Preparation

About 40 mg of composite placebo powder was accurately weighed and quantitatively transferred into a 250-mL volumetric flask. 12.5 mL of 1N Sodium Hydroxide solution was added and the flask was swirled. The solution was allowed to stand for 24 hours at ambient conditions. The solution was neutralized with 12.5 mL of 1 N Hydrochloric Acid solution. The flask was filled with diluent to ¾ of flask volume and the flask was swirled to avoid clumping. The flask was sonicated for 15 minutes with occasional swirling, and mechanically shaken for 15 minutes. The solution was allow to cool to room temperature, then diluted to volume with diluent and mixed well. An aliquot of the placebo solution was centrifuged at 10000 rpm (11400 RCF) for 10 minutes.

6.6.3 Base Hydrolysis Sample Solution Preparation

About 86 mg of tablet powder was accurately weighed and quantitatively transferred into a 250-mL volumetric flask. 12.5 mL of 1 N Sodium Hydroxide was added and the flask was swirled. The solution was allowed to stand for 24 hours at ambient conditions. Then, the solution was neutralized with 12.5 mL of 1 N Hydrochloric Acid solution. The flask was filled with diluent to ¾ of flask volume and swirled to avoid clumping. The flask was sonicated for 15 minutes with occasional swirling, and mechanically shaken for 15 minutes. The solution was allowed to cool to room temperature, then diluted to volume with diluent and mixed well. An aliquot of the sample solution was centrifuged at 10000 rpm (11400 RCF) for 10 minutes.

6.7 Oxidation by Peroxide (5% Hydrogen Peroxide)

6.7.1 ~5% Hydrogen Peroxide Preparation

16.5 mL of concentrated hydrogen peroxide was diluted to 100 mL with purified water.

6.7.2 Peroxide Oxidation Blank Preparation

5.0 mL of ~5% hydrogen peroxide solution was diluted to 100 mL with diluent. Mix well.

6.7.3 Peroxide Oxidation Placebo Solution Preparation

About 40 mg of composite placebo powder was accurately weighed and quantitatively transferred into a 250-mL volumetric flask. 12.5 mL of 5% hydrogen peroxide solution was added and the flask was gently swirled. The solution was allowed to stand for at least 24 hours at ambient condition. The flask was filled with diluent to ¾ of flask volume and swirled to avoid clumping. The flask was sonicated for 15 minutes with occasional swirling, and mechanically shaken for 15 minutes. The solution was allowed to cool to room temperature, then diluted to volume with diluent and mixed well. An aliquot of the placebo sample solution was centrifuged at 10000 rpm (11400 RCF) for 10 minutes.

6.7.4 Peroxide Oxidation Sample Solution Preparation

About 86 mg of tablet powder was accurately weighed and quantitatively transferred into a 250-mL volumetric flask. 12.5 mL of 5% hydrogen peroxide solution was added and the flask was gently swirl. The solution was allowed to stand for at least 24 hours at ambient condition. The flask was filled with diluent to ¾ of flask volume and swirled to avoid clumping. The flask was sonicated for 15 minutes with occasional swirling, and mechanically shaken for 15 minutes. The solution was allowed to cool to room temperature, then diluted to volume with diluent and mixed well. An aliquot of the sample solution was centrifuged at 10000 rpm (11400 RCF) for 10 minutes.

6.8 Results and Discussion

All system suitability requirements were met.

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

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 (11%) when exposed to the peroxide oxidation condition for approximately

24 hours. No degradation peaks, placebo or otherwise related to CX-4945, were found to significantly interfere with the CX-4945 peak.

There was no significant degradation found at the exposed acid and base hydrolytic, thermal (ambient, elevated temperature), and UV photolytic degradation conditions. The drug product was found to have some susceptibility to degradation at the maximum base hydrolytic condition. Despite exposure to the maximum, most aggressive, base hydrolytic degradation condition, the degradation was <5%, suggesting that the drug product is unlikely to degrade via this mechanism of degradation at more practical, reasonable conditions.

For all the 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
(Notebook Reference: ARD-0378, pg. 78, 86, 89)

Sample Condition	Recovery Against Control, %	% Degradation	Resolution	Purity Angle	Purity Threshold
Control 1	N/A	—	17.7	0.317	3.747
Control 2	N/A	—	17.3	0.312	3.866
Ambient	103	—	18.0	0.337	3.972
Elevated Temperature	99	—	18.3	0.295	3.631
Photolytic	99	—	18.2	0.289	3.573
Acid Hydrolysis	100	—	18.3	0.296	3.864
Base Hydrolysis	97	3	7.2	0.269	3.515
Oxidation (H_2O_2)	89	11	8.6	0.212	2.646

Acceptance Criteria:

- Degradation should be between 5% to 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).

Figures 6 and **7** are the chromatograms of the placebo and sample after exposing to ambient conditions.

Figures 8 and **9** are the chromatograms of the placebo and sample after exposing to elevated temperature.

Figures 10 and **11** are the chromatograms of the placebo, sample after exposing UV photolytic conditions.

Figure 12 are the chromatograms of the acid/blank solution

Figures 13 and **14** are the chromatograms of the placebo and sample after exposing to acid hydrolytic conditions.

Figures 15, and **16** are the chromatograms of the placebo and sample after exposing to base hydrolytic conditions.

Figures 17, **18**, and **19** are the chromatograms of the oxidation blank solution, placebo and sample after exposing to oxidation conditions.

Based on the results from the study, Specificity (by forced degradation) of the method was demonstrated.

7 LINEARITY STUDY

The linearity study was performed on CX-4945 at appropriate range for Assay and Impurities.

For assay, the linearity was assessed over the intended range of method of 50% to 150% of the nominal active concentration of 0.2 mg/mL (0.1 mg/mL to 0.3 mg/mL).

For related substances, the linearity was assessed from the QL level (0.05%) to 0.3% of the nominal active concentration of 0.2 mg/mL (0.1 µg/mL to 0.6 µg/mL).

7.1 Assay Linearity Stock Solution Preparation

The Stock Standard Solution was prepared as directed in **Section 2.6.1**.

7.2 Impurity Linearity Stock Solution Preparation

The intermediate sensitivity solution was prepared as directed in **Section 2.7**.

7.3 Assay Linearity Working Solutions Preparation

The linearity solutions for the L1 to L5 levels were prepared as directed in **Table 7-1**. Each flask was diluted to volume with diluent and mixed well.

Table 7-1. Assay Linearity Solutions Preparation

Assay Linearity Level	Nominal Conc. (%)	Volume of Assay Linearity Stock Solution (mL)	Flask Volume (mL)	Approx. Conc. of CX-4945 (mg/mL)
L1	50	5.0	50	0.1
L2	75	7.5	50	0.15
L3	100	10.0	50	0.2
L4	125	12.5	50	0.25
L5	150	15.0	50	0.3

7.4 Impurity Linearity Working Solution

The working linearity solutions for the QL to L5 levels were prepared as directed in **Table 7-2**. Dilute each to volume with diluent and mix well.

Table 7-2. Impurity Linearity Solutions Preparation

Impurity Linearity Level	Nominal Conc. (%)	Volume of Impurity Linearity Stock Solution (mL)	Flask Volume (mL)	Approx. Conc. of CX-4945 (µg/mL)
L1	QL	2.5	100	0.1
L2	0.1	5.0	100	0.2
L3	0.15	7.5	100	0.3
L4	0.2	10.0	100	0.4
L5	0.3	15.0	100	0.6

7.5 Results and Discussion

All system suitability requirements were met.

For CX-4945 Assay level:

The linearity results for CX-4945 at the Assay level are summarized in **Table 7-3**. All acceptance criteria of the study were met.

The CX-4945 linearity was demonstrated from about 0.1 mg/mL to 0.3 mg/mL, which corresponds to 50% to 150% of the nominal CX-4945 concentration in standard and sample solution of 0.2 mg/mL.

Figure 20 is a plot of the mean area response vs. concentration.

Table 7-3. Linearity Results for CX-4945 (at the Assay level)
(Notebook Reference: ARD-0379, pg. 92-93)

Assay Level	Nominal Conc. %	Concentration, µg/mL	Area	Response Factor	Relative Response Factor, %
L1	50	100.77444	1625812.6193	16133.1843	100.8
L2	75	151.16166	2444055.3980	16168.4874	101.0
L3	100	201.54888	3226612.6995	16009.0827	100.0
L4	125	251.93610	4023155.5620	15968.9522	99.7
L5	150	302.32332	4839134.0704	16006.4862	100.0
Slope				15,888.43970	
Y-Intercept				29,456.84336	
Y-Intercept relative to the mean peak area response at 100% level				0.9%	
Correlation Coefficient, r				1.000	

Acceptance Criteria:
- Meet the linearity range of at least five consecutive levels.
- The correlation factor, r, is NLT 0.999.
- The linearity relative response factor (RRF) at each level is within 98.0% to 102.0%.

For Impurity level:

The linearity results for CX-4945 at the Impurity level are summarized in **Table 7-4**. All acceptance criteria of the study were met.

The linearity of CX-4945 was demonstrated from about 0.1 µg/mL to 0.6 µg/mL, which corresponds to an impurity level of 0.05% to 0.3% with respect to a nominal CX-4945 concentration in the sample solution of 0.2 mg/mL.

Figure 21 is a plot of the mean area response vs concentration.

Table 7-4. Linearity Results for CX-4945 (at Impurity level)
(Notebook Reference: ARD-0379, pg. 94-96)

Assay Level	Nominal Conc. %	Concentration, µg/mL	Area	Response Factor	Relative Response Factor, %
L1	QL	0.1075308	2008.7700	18680.8802	116
L2	0.1	0.2150616	3432.7408	15961.6630	99
L3	0.15	0.3225924	5213.2115	16160.3667	100
L4	0.2	0.4301232	7004.9070	16285.8153	101
L5	0.3	0.6451848	10558.3039	16364.7747	101
Slope				16,077.07271	
Y-Intercept				111.48907	
Y-Intercept relative to the mean peak area response at 100% level				2.1%	
Correlation Coefficient, r				0.999	
Relative Slope of Impurity level to Assay level				101%	

Acceptance Criteria:
- Meet the linearity range of a minimum of five consecutive levels.
- The linearity relative response factors (RRF) at each level is within 80% to 120%.
- The relative slope of impurity level to assay level is within 90% to 110%.
- For the impurities level linearity, the correlation coefficient, r, is NLT 0.995.

8 QUANTITATION LIMIT

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

8.1 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 8-1.** All criteria were met.

The QL of the method was demonstrated at an impurity level of 0.05%, which corresponds to an impurity concentration of 0.1 µg /mL.

Figure 22 is a chromatogram of the working QL solution (sensitivity solution).

Table 8-1: S/N of CX-4945 peak in the sensitivity solution.
(Notebook Reference: ARD-0379, pg. 97)

Item	Injection #	Area Response	S/N
Sensitivity solution (QL)	1	1916.6206	16
	2	1867.0609	35
	3	1833.9617	27
	4	1969.1865	21
	5	1812.2829	32
	6	1975.7645	26
RSD (%)		3.6	

Acceptance Criteria:
- The S/N is NLT 10 in each injection.
- The % RSD of peak area responses is NMT 15% for the active.

9 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 tablet composite placebo.

For Assay, the accuracy was evaluated from CX-4945 concentrations corresponding to 50% to 150% of the nominal sample concentration of 0.2 mg/mL.

For Impurities, the accuracy was evaluated from concentrations corresponding to an impurity level of 0.05% to 0.3% of the nominal sample concentration of 0.2 mg/mL.

9.1 Accuracy for Assay

9.1.1 Recovery Sample Preparations

About 100 mg of composite placebo and CX-4945 sodium salt drug substance were accurately weighed as directed in **Table 9-1** into 100-mL volumetric flasks. About ¾ volume of diluent was added and the flasks were swirled to avoid clumping. The flasks were sonicated for 15 minutes with occasional swirling, and mechanically shaken for 15 minutes. The solutions were allowed to cool to room temperature, then diluted to volume with diluent and mixed well. An aliquot of the solution was filtered through a Millipore 0.45-µm PVDF membrane filter, discarding the first 3 mL to waste.

5.0 mL of the filtrate was diluted to 50 mL with diluent and mixed well.

Each level was prepared in triplicate.

Table 9-1. 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	Approx. Conc. CX-4945 * (mg/mL)
R1	50%	125	100	100	5.0 mL to 50 mL	0.1
R2	100%	250	100	100		0.2
R3	150%	375	100	100		0.3

*Approximate concentration based on CX-4945 sodium salt containing ~15% water.

9.2 Control/Reference Solution Preparation

9.2.1 Stock Sample Solution Preparation:

An amount of sample equivalent to 200 mg of CX-4945 in the free acid form (approximately 250 mg of CX-4945 as sodium salt) was accurately weighed and quantitatively transferred into a 100 mL volumetric flask. About ¾ volume of diluent was added and the flask was swirled to dissolve the drug substance. The flask was sonicated until completely dissolved. The solution was allowed to cool to room temperature, then diluted to volume with diluent and mixed well.

A check stock sample solution was prepared in a similar manner.

9.2.2 Working Sample Solution Preparation:

5.0 mL of the stock sample solution was diluted to 50 mL with the diluent, and mixed well.

The concentration of CX-4945 free acid was about 0.2 mg/mL.

A working check sample solution was prepared in a similar manner.

Note—The water content of the CX-4945 sodium salt drug substance was determined as per the analytical procedure in method verification protocol PRO MV 0129.

9.3 Accuracy for Related Substances

9.3.1 Spiking Solution Preparation

About 125 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. About ¾ volume of diluent was added and the flask was swirled to dissolve the drug substance. The flask was sonicated until completely dissolved. The solution was allowed to cool to room temperature, then diluted to volume with diluent and mixed well.

5.0 mL of the above solution was diluted to 100 mL with diluent and mixed well.

The concentration of CX-4945 free acid was 0.05 mg/mL.

9.3.2 Recovery Sample Preparations

About 100 mg of CX-4945 tablet composite placebo was accurately weighed into 100-mL volumetric flasks. Volumes of recovery spiking solution were transferred as directed in **Table 9-2**. About ¾ volume of diluent was added and the flask was swirled to avoid clumping. The flask was sonicated for 15 minutes with occasional swirling, and mechanically shaken for 15 minutes. The solution was allowed to cool to room temperature, then diluted to volume with diluent and mixed well. An aliquot of the solution was filtered through a Millipore 0.45-µm PVDF membrane filter, discarding the first 3 mL to waste.

5.0 mL of the filtrate was diluted to 50 mL with diluent and mixed well.

Table 9-2. Preparation of Recovery sample solutions for Impurities

Recovery Level	Impurity Level (%)	Volume of Spiking Solution (mL)	Weight of Placebo (mg)	Flask Volume (mL)	Dilution	Approx. Conc. of CX-4945 (µg/mL)
R1 (QL)	0.05	2.0	100	100	5.0 mL to 50 mL	0.1
R2	0.1	4.0	100	100		0.2
R3	0.15	6.0	100	100		0.3
R4	0.3	12.0	100	100		0.6

Each level was prepared in triplicate.

9.4 Control/Reference Solution Preparation

6.0 mL of the spiking solution (**Section 9.3.1**) was diluted to 100 mL with the diluent and mixed well.

5.0 mL of the above solution was diluted to 50 mL with the diluent and mixed well.

Note—The water content of the CX-4945 sodium salt drug substance was determined as per the analytical procedure in method verification protocol PRO MV 0129.

9.5 Results and Discussion

All system suitability requirements were met. Triplicate sample solutions were prepared for each recovery level and each sample solution was injected once.

9.5.1 Accuracy for Assay

The accuracy study results for CX-4945 at the Assay level are summarized in **Table 9-3**. All acceptance criteria were met.

Accuracy for assay of the method was demonstrated from about 0.1 mg/mL to 0.3 mg/mL, which corresponds to 50% to 150% of the specification.

Table 9-3. Recovery results for assay
(Notebook Reference: ARD-0379, pg. 98)

Recovery Level	Weight of CX-4945 Sodium Salt (mg)	Recovery (%)	Mean Recovery (%)	RSD (%)
R1 (50%)	125.8	100.1943	100.2	0.7
	125.0	99.4858		
	125.3	100.9379		
R2 (100%)	250.6	100.8573	100.7	0.5
	251.3	100.1229		
	250.7	101.1936		
R3 (150%)	376.3	98.9930	99.3	0.3
	377.6	99.6044		
	378.3	99.2348		

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%.

9.5.2 Accuracy for Impurities

The accuracy study results for CX-4945 at the Impurity level are summarized in **Table 9-4**. All acceptance criteria were met.

Accuracy for Impurities of the method was demonstrated from about 0.1 µg/mL to 0.6 µg/mL, which corresponds to 0.05% to 0.3% of the impurity level with respect to nominal CX-4945 concentration of sample solution.

Table 9-4. Recovery results for impurities
(Notebook Reference: ARD-0379, pg. 99)

Recovery Level	Weight of CX-4945 used for Spiking Solution (mg)	Recovery (%)	Mean Recovery (%)	RSD (%)
R1 (QL) – 0.05%	126.0	100.3040	102	1.3
		102.3295		
		102.6660		
R2 (0.1%)		85.6640	95	8.3
		99.4110		
		99.0575		
R3 (0.15%)		97.7972	98	0.9
		97.9709		
		99.4105		
R4 (0.3%)		97.1155	98	0.8
		97.6727		
		98.6968		

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%.

10 PRECISION AND INTERMEDIATE PRECISION

10.1 Precision

10.1.1 Precision – Assay

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

10.1.2 Precision – Content Uniformity

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

10.1.3 Precision – Related Substances

Six (6) sample solutions were prepared at the R3 level as described in **Section 8.3.2**.

10.2 Intermediate Precision

10.2.1 Intermediate Precision – Assay

Similarly, as directed in the precision study, an additional six (6) sample solutions were prepared as directed in **Section 2.10** and analyzed by a different analyst using a different HPLC system and column.

10.2.2 Intermediate Precision – Content Uniformity

Similarly, as directed for the precision study, an additional ten (10) sample solutions were prepared as directed in **Section 2.11** and analyzed by a different analyst using a different HPLC system and column.

10.3 Results and Discussion

All system suitability requirements were met for both the Precision and Intermediate Precision studies. All solutions were injected once.

For Assay:

The results from the precision and intermediate precision studies are summarized in **Table 10-1**. All acceptance criteria were met.

Table 10-1. Precision Results for Assay

(Notebook Reference: ARD-0378, pg. 62-63, ARD 0379, pg. 109)

	Sample	Assay, %	Retention Time, min	Standard Retention Time (from system suitability standards)
Precision	1	96.6023	6.1630	RT mean: 6.1838 min RT range: 6.0405 min – 6.2871 min
	2	95.9929	6.1638	
	3	95.8874	6.1579	
	4	96.3016	6.1619	
	5	96.3547	6.1649	
	6	96.3618	6.1587	
	Mean	**96.2501**		
	RSD, %	**0.3**		
Intermediate Precision	1	97.0123	5.5352	RT mean: 5.5411 min RT range: 5.4303 min – 5.6519 min
	2	95.9163	5.5346	
	3	96.2425	5.5347	
	4	97.8004	5.5320	
	5	97.6798	5.5353	
	6	96.9977	5.5308	
	Mean	**96.9415**		
	RSD, %	**0.8**		
% Difference of Mean		**0.7**		

Acceptance Criteria:

- The RSD of the results from the Precision study (n=6) is NMT 3.0%.
- The RSD of the results from the Intermediate Precision study (n=6) is NMT 3.0%.
- The absolute difference between the mean results obtained in the precision and intermediate precision studies is NMT 3.0%.
- The retention time of CX-4945 in each sample solution corresponds to that of the standard solution within ±2.0%.

For Content Uniformity:

The results from the precision and intermediate precision studies are summarized in **Table 10-2**. All acceptance criteria were met.

Table 10-2. Precision and Intermediate Precision results for Content Uniformity

(Notebook Reference: ARD-0378, pg. 64; ARD 0379, pg. 110)

	Sample	Precision % Label Claim	Intermediate Precision % Label Claim
Precision	1	95.06	97.04
	2	95.63	96.91
	3	96.45	97.54
	4	96.45	97.62
	5	95.63	97.23
	6	95.98	97.41
	7	97.48	96.51
	8	96.83	93.92
	9	96.94	97.54
	10	95.62	97.84
	Mean	**96.2 (96.20)**	**97.0 (96.95)**
	AV	**4.1**	**4.3**
Absolute Difference of Mean, %		**0.8**	

Acceptance Criteria:

- The acceptance value (AV) is NMT 15.0.
- The absolute difference between the mean results obtained in the precision and intermediate precision studies is NMT 3.0%.

For related Substances:

The results from the precision study are summarized in **Table 10-3**. All acceptance criteria were met.

Table 10-3. Precision results for Impurities

(Notebook Reference: ARD-0379, pg. 100)

Sample	% Impurity	Absolute Difference, %
1	0.1575	0.001
2	0.1578	0.001
3	0.1601	0.002
4	0.1567	0.002
5	0.1614	0.003
6	0.1568	0.002
Mean	**0.1583**	
RSD, %	**1.2**	

Acceptance Criteria:

• The % RSD of the impurity results ≥ 0.6% from Precision studies (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. See table below:

% Related Substance	Absolute Difference
≥ 0.05 and ≤ 0.30	NMT 0.10
> 0.30 and < 0.6	NMT 0.20

11 FILTER STUDY

A filter study was performed to evaluate the suitability of the filters used (Millipore 0.45-µm PVDF membrane filter) for the sample solution preparation of Assay methods.

11.1 Filter Study on Diluent

A portion of the diluent was filtered through a Millipore 0.45-µm PVDF filter and the first 3 mL of filtrate were collected.

2.5 mL of the filtrate was diluted to 25 mL with the diluent, and mixed well.

11.2 Filter Study on Assay Sample Solution

Filtered Sample:

A portion of the assay sample solution prepared as per **Section 2.9** was filtered (Note—A sample solution prepared for **Section 8.1** may be used) through a Millipore 0.45-µm PVDF filter, and each aliquot portion was collected as shown in **Table 11-1**.

Table 11-1. 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

2.5 mL of the filtrate was diluted to 50 mL with the diluent, and mixed well.

Centrifuged Sample:

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

2.5 mL of the supernatant was diluted to 50 mL with the diluent, and mixed well.

11.3 Filter Study on Related Substance Sample Solution

Filtered Sample:

A portion of the related substance sample solution (prepared as per **Section 8.3**) was filtered through a Millipore 0.45-µm PVDF filter, and each aliquot portion was collected as shown in **Table 11-1**.

2.5 mL of the filtrate was diluted to 50 mL with the diluent, and mixed well.

Centrifuged Sample:

Centrifuge an aliquot of the sample solutions evaluated for the filter study at 10000 rpm for 10 minutes.

2.5 mL of the supernatant was diluted to 50 mL with the diluent, and mixed well.

11.4 Results and Discussion

All system suitability requirements were met.

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 11-2**. All criteria were met.

Table 11-2. Results from filter study on sample solution - Assay
(Notebook Reference: ARD-0379, pg. 111)

Filter	Aliquot	Filtration Fraction (mL)	Recovery (%)	Relative Recovery (%)
Centrifuge	—	—	96.2222	—
Millipore 0.45 µm PVDF	1	0-3	95.9202	99.7
	2	3-6	96.1352	99.9
	3	6-9	96.6302	100.4

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 11-3**.

Table 11-3. Results from filter study on sample solution - Related Substances
(Notebook Reference: ARD-0379, pg. 101)

Filter	Aliquot	Filtration Fraction (mL)	Recovery (%)	Relative Recovery (%)
Centrifuge	—	—	104.4037	—
Millipore 0.45 µm PVDF	1	0-3	82.1845	78.7
	2	3-6	97.2719	93.2
	3	6-9	97.6351	93.5

Acceptance Criteria:

• For related substances, the percent recovery of CX-4945 in each filtrate aliquot of the sample solution to the centrifuged sample solution is within 90.0% – 110.0%.

The acceptance criteria was not met for the aliquot portion of 0-3 mL filtrate. However, the acceptance criteria was met at aliquots 3-6 mL and 6-9 mL which indicates that a discard volume of 3 mL is appropriate.

Based on study findings, the Millipore 0.45-µm PVDF filters were found to be suitable for the sample preparation with a discard volume of at least 3 mL.

12 STABILITY STUDY

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

Sample solution stability is considered from the time of initial injection to the time of injection of the aged solution.

12.1 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 12-1**.

All criteria were met at each evaluated interval.

The working standard solution was found to be stable for at least 6 days when stored at normal laboratory environmental condition.

Table 12-1. Results from the stability study of the working standard solution

Time	Recovery, (%)	Relative Recovery, (%)	Reference
Initial	99.5485	—	ARD-0379, pg. 90
Day 1	99.6909	100.1	ARD-0379, pg. 108
Day 2	100.0345	100.5	ARD-0379, pg. 117
Day 5	99.0269	99.5	ARD-0379, pg. 129
Day 6	100.5712	101.0	ARD-0379, pg. 135

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 – Drug Product:

The sample solution for drug product stability results are summarized in **Table 12-2**.

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

Table 12-2. Results from the stability study of the sample solution - Drug Product

Time	Recovery, (%)	Relative Recovery, (%)	Reference
Initial	96.6023	—	ARD-0379, pg. 109
Day 1	95.2020	98.6	ARD-0379, pg. 117
Day 4	95.2152	98.6	ARD-0379, pg. 129
Day 5	96.0076	99.4	ARD-0379, pg. 135

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 if present in the sample solution ≥ 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 if present in the sample solution ≥ 0.4%, the relative % impurity in the aged sample solution to the initial sample solution is within 85.0% – 115.0%.

13 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 10.1.1**). The successful establishment and completion of these studies are considered fulfillment of Identification by RT.

13.1 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 are summarized in **Table 10-1**. All criteria were met.

14 CONCLUSION

The method verification protocol PRO MV 0176-2 was successfully executed. The study findings are summarized below. Based on the findings, the CX-4945 (Silmitasertib) Tablets, 500 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.
- 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 ambient, elevated temperature, photolytic, acid hydrolysis and base hydrolysis conditions. For all 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 0.1 µg/mL.
- Accuracy: Accuracy for *Assay*/*Blend Uniformity*/*Content Uniformity* was demonstrated from about 0.1 mg/mL to 0.3 mg/mL, which corresponds to 50% to 150% of the specification. Accuracy for *Related Substances* was demonstrated from about 0.1 µg/mL to 0.6 µg/mL, which corresponds to an impurity level of 0.05% to 0.3% of the specification (or 33.3% to 200% of the impurity specification).
- Precision and Intermediate Precision: The precision and Intermediate Precision of the method was successfully demonstrated for *Assay*, *Blend Uniformity*, *Content Uniformity*, *Related Substances*, and *Identification by RT*.
- 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 at least 3 mL.
- Stability of the Standard Solution: The working standard solution was found to be stable for at least 6 days when stored at normal laboratory environmental condition
- Stability of the Sample Solution: The stability of the sample solution was established as 5 days stored at normal laboratory environmental condition.

15 FIGURES

Figure 1. A representative chromatogram of the working standard solution

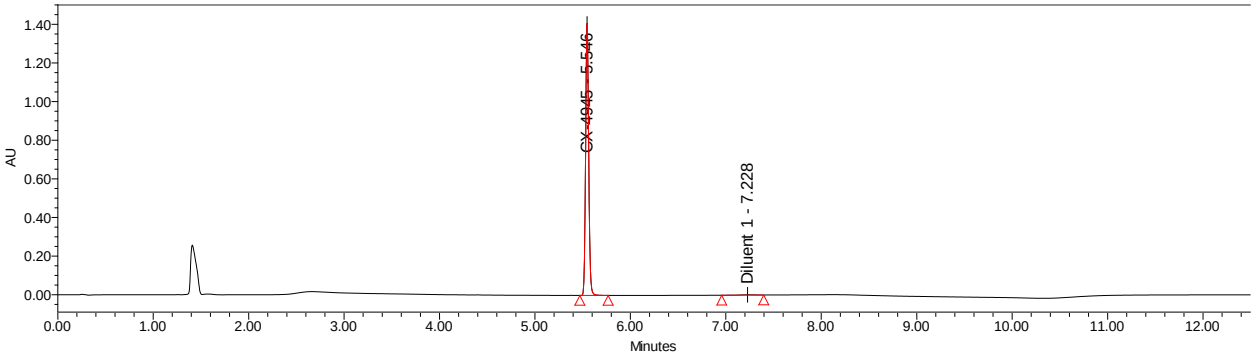


Figure 1a. An expanded chromatogram of the working standard solution

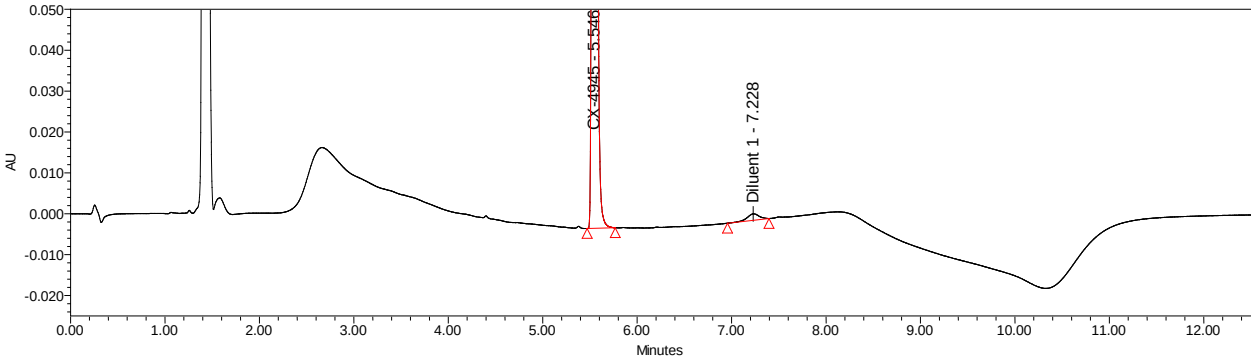


Figure 2. A representative chromatogram of the diluent interference solution

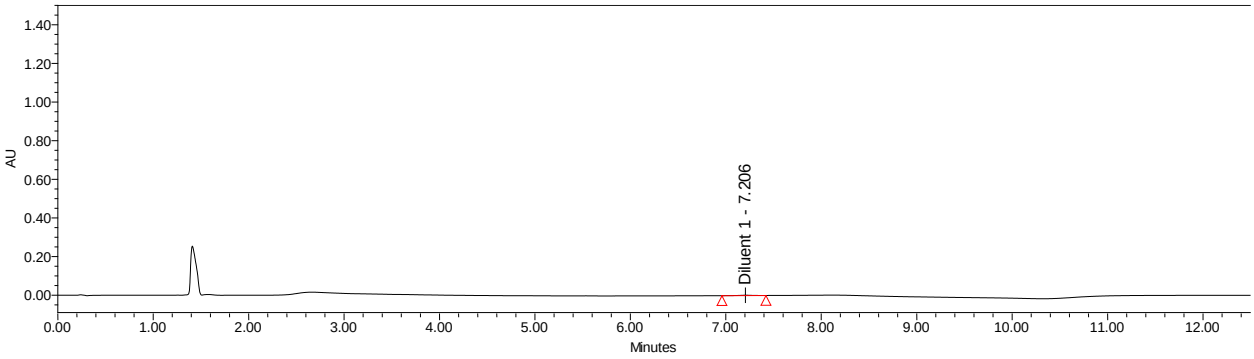


Figure 2a. An expanded chromatogram of the diluent interference solution

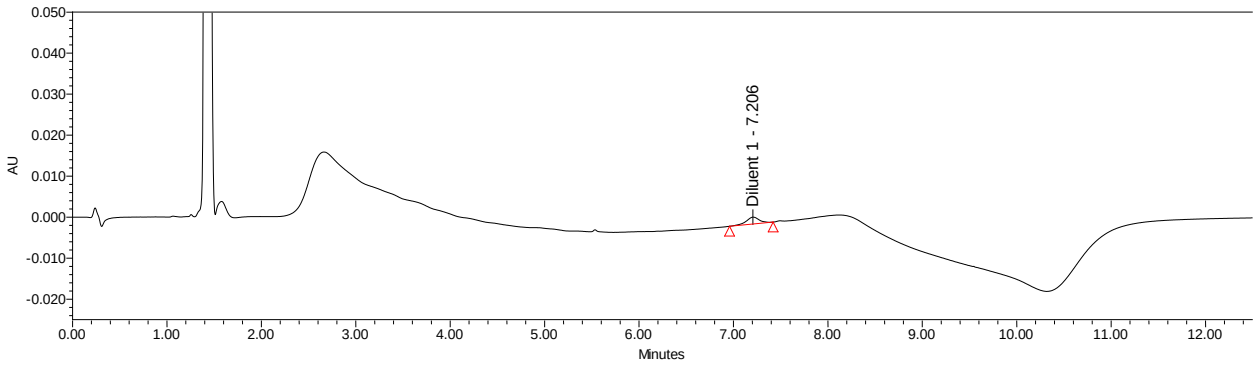


Figure 3. A representative chromatogram of the placebo interference solution

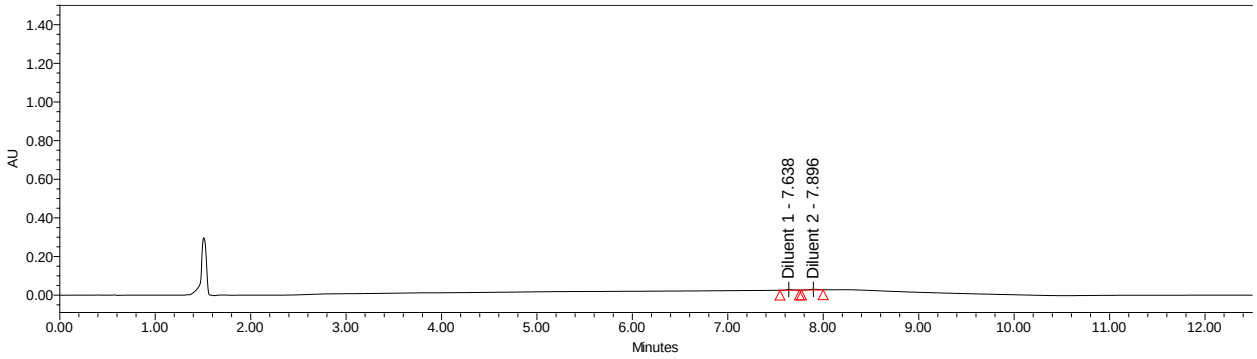


Figure 4. A representative chromatogram of the CX-4945 Impurity 1 working ID solution

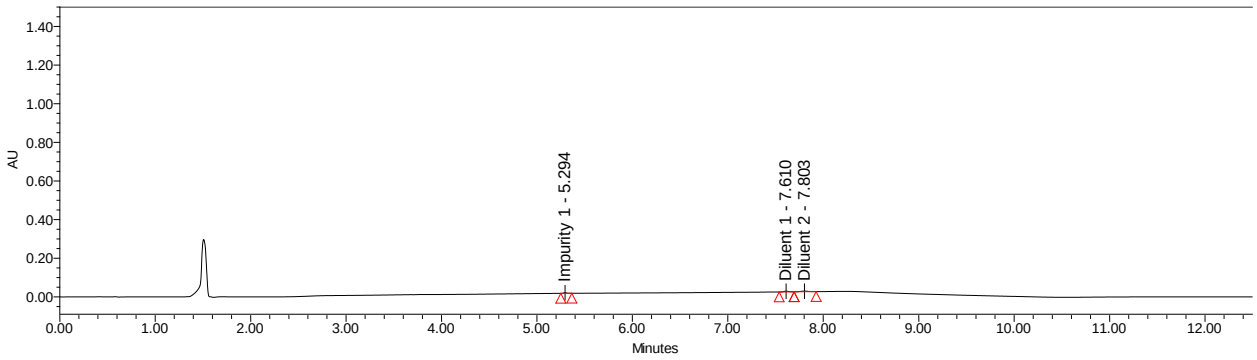


Figure 5. A representative chromatogram of the CX-4945 Impurity 2 working ID solution

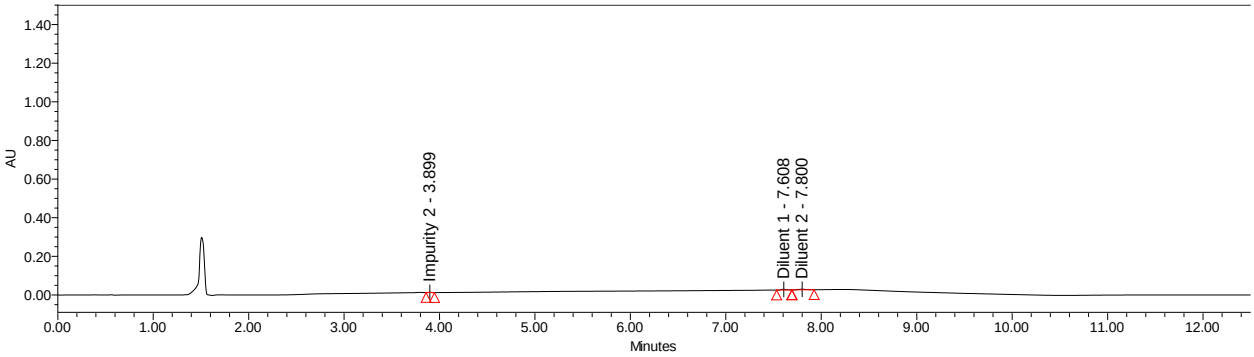


Figure 6. A representative chromatogram of the placebo solution after exposing to ambient conditions

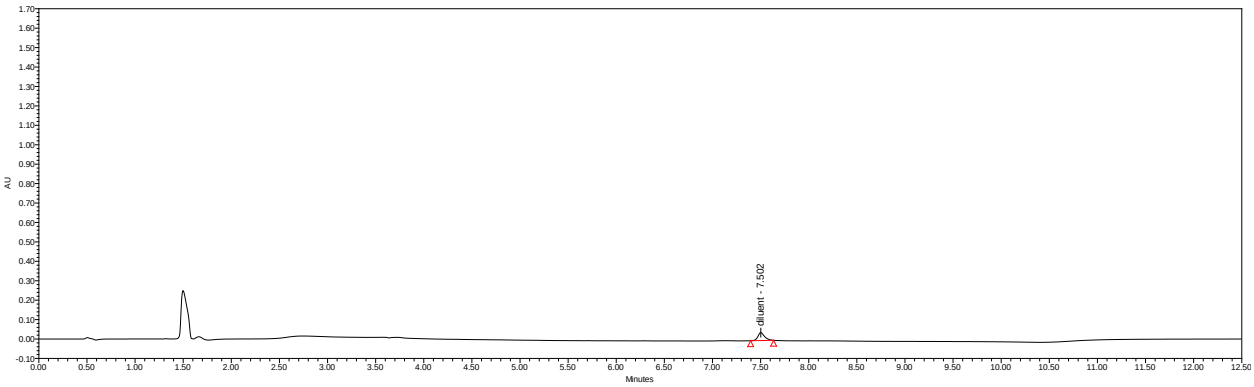


Figure 7. A representative chromatogram of the sample solution after exposing to ambient conditions

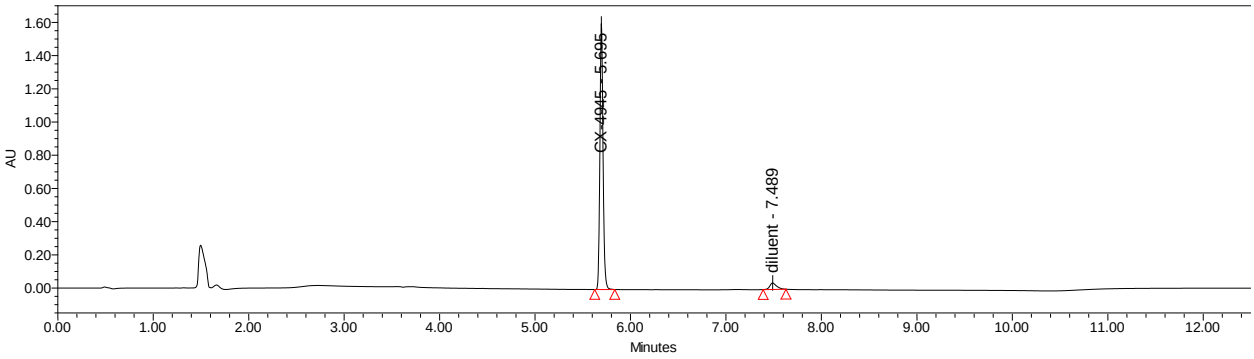


Figure 8. A representative chromatogram of the placebo solution after exposing to elevated temperature

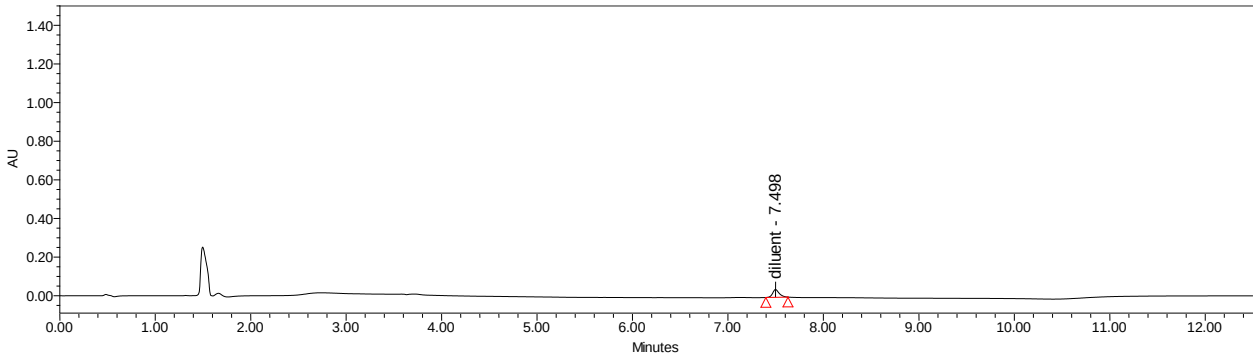


Figure 9. A representative chromatogram of the sample solution after exposing to elevated temperature

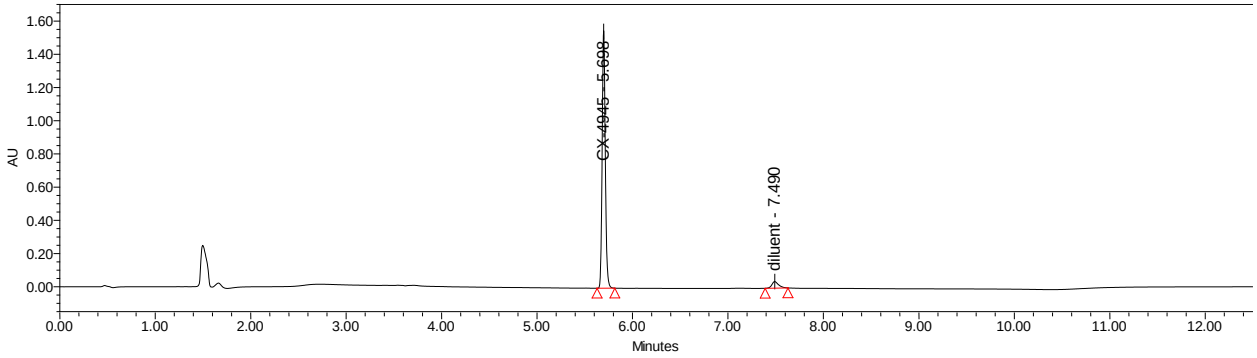


Figure 10. A representative chromatogram of the placebo solution after exposing to UV photolytic conditions

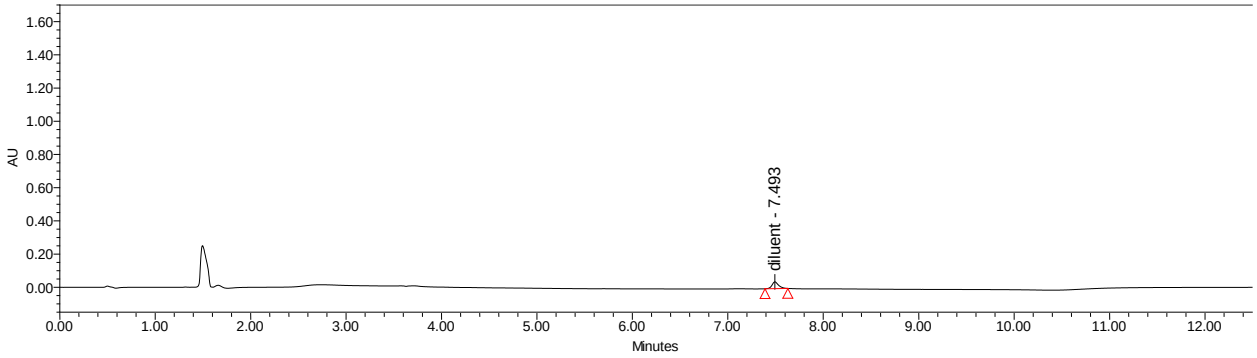
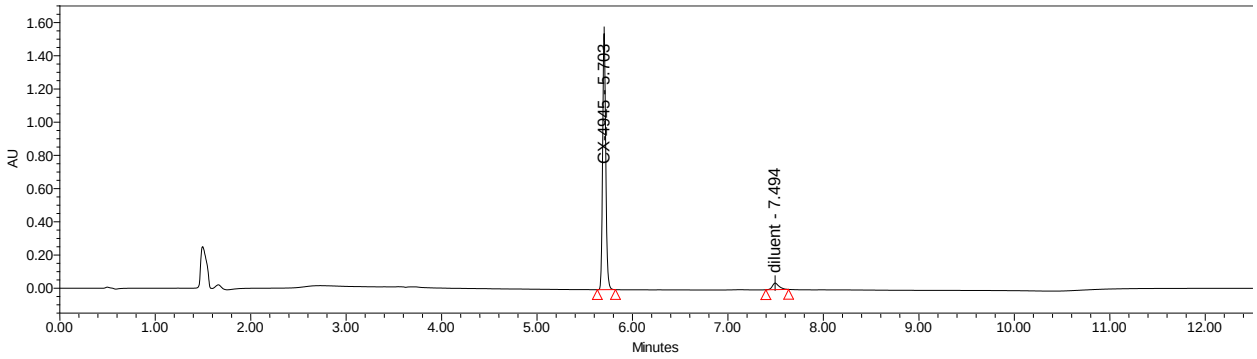


Figure 11. A representative chromatogram of the sample solution after exposing to UV photolytic conditions



Figures 12. A representative chromatogram of the acid/base blank solution

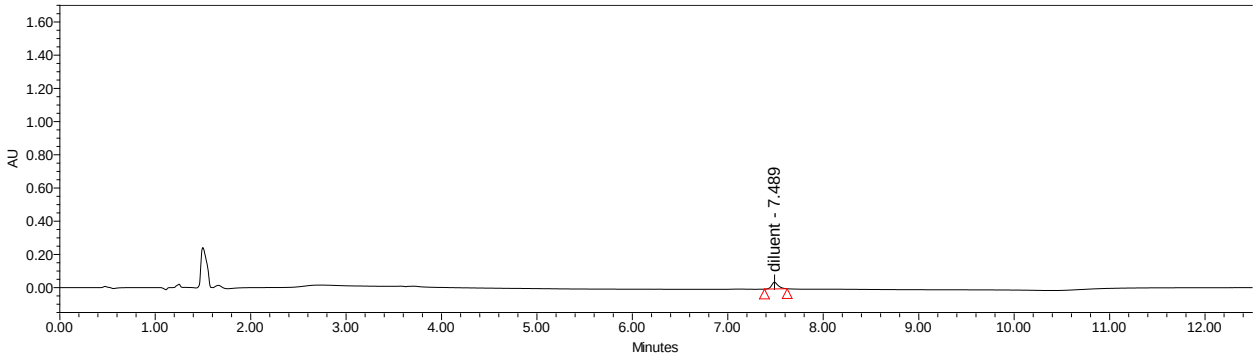


Figure 13. A representative chromatogram of the placebo after exposing to acid hydrolytic conditions

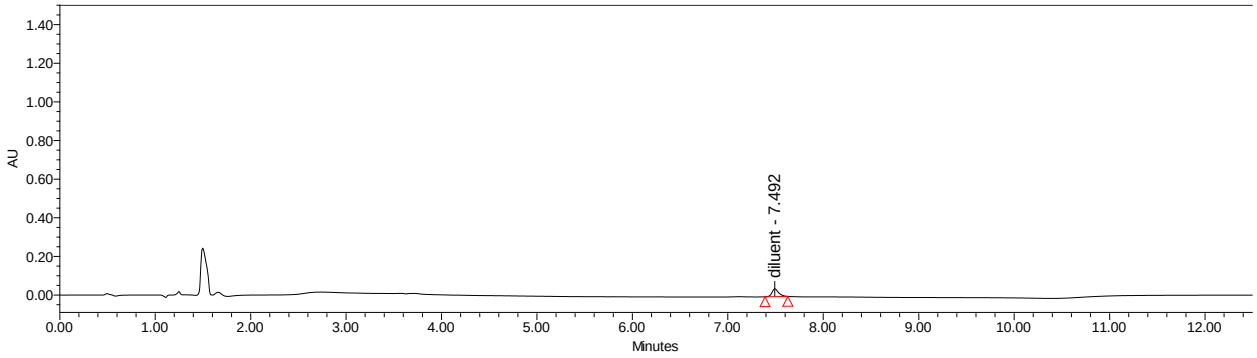


Figure 14. A representative chromatogram of the sample after exposing to acid hydrolytic conditions

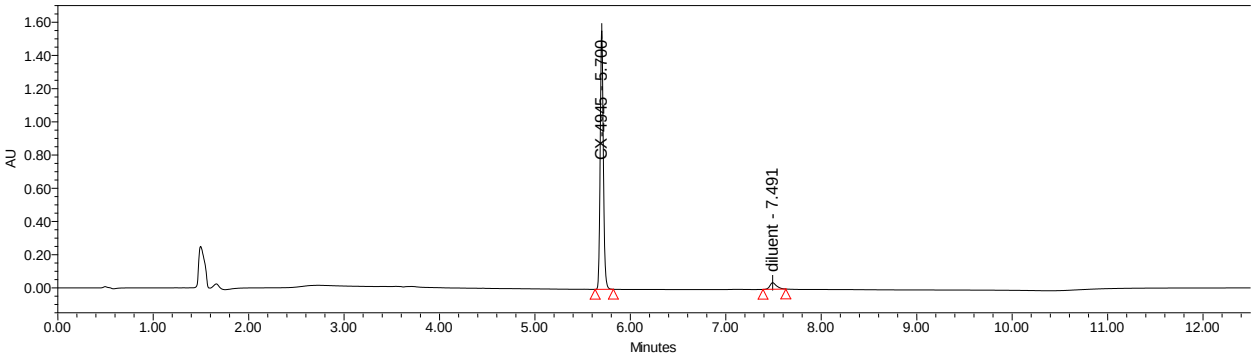


Figure 15. A representative chromatogram of the placebo after exposing to base hydrolytic conditions

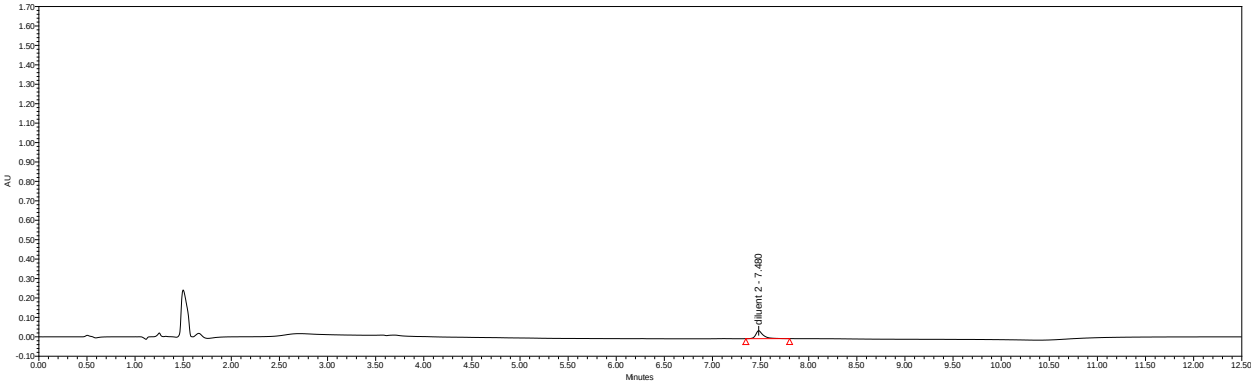
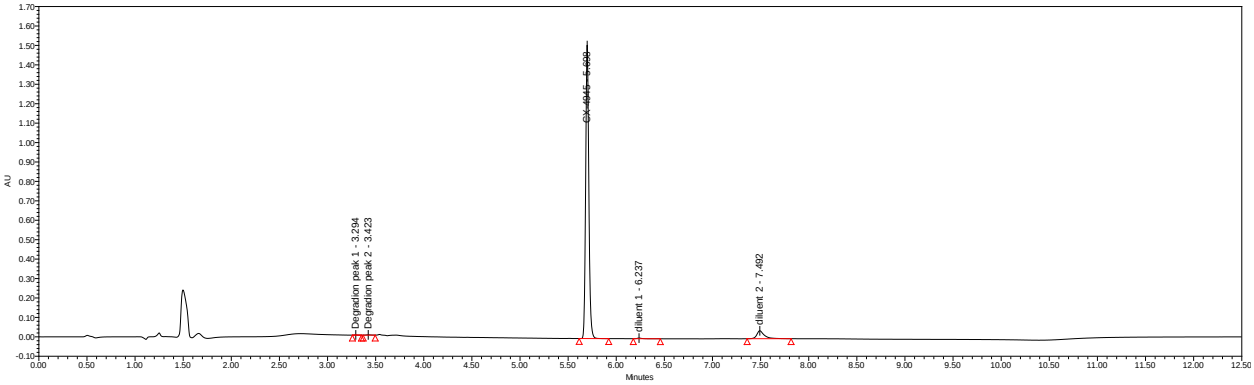


Figure 16. A representative chromatogram of the sample after exposing to base hydrolytic conditions



Figures 17. A representative chromatogram of the oxidation blank solution

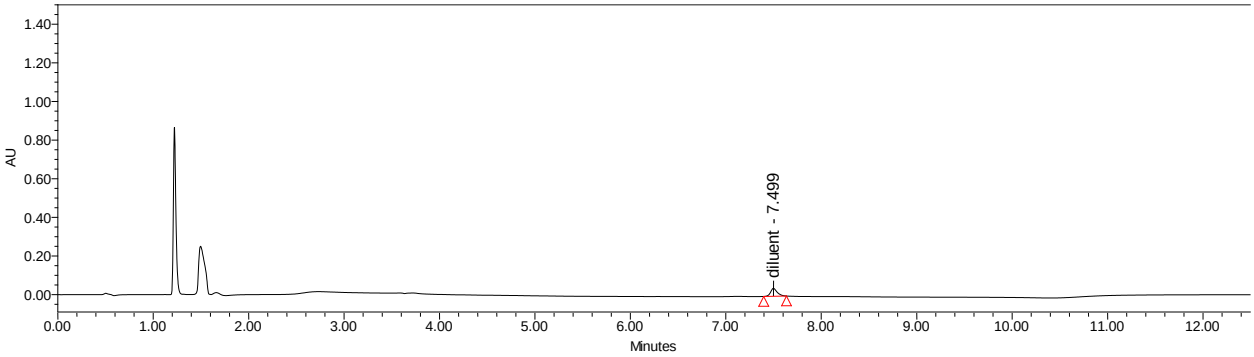


Figure 18. A representative chromatogram of the placebo after exposing to oxidation conditions

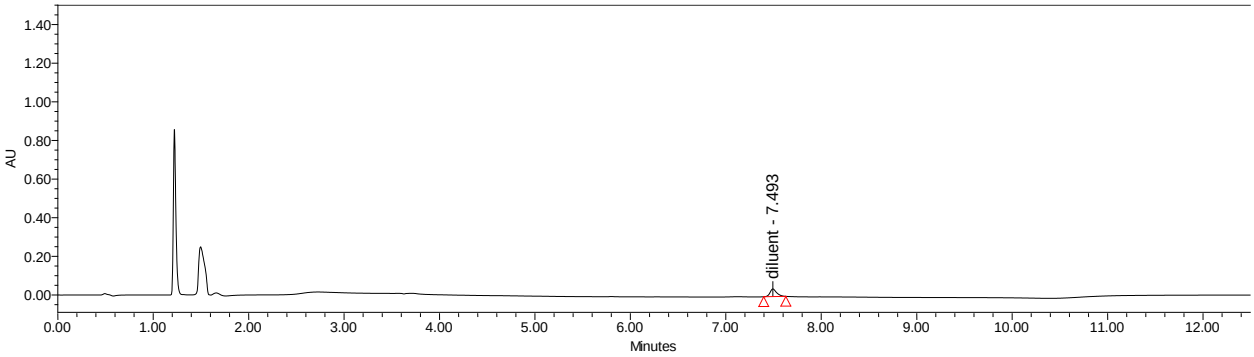


Figure 19. A representative chromatogram of the sample after exposing to oxidation conditions

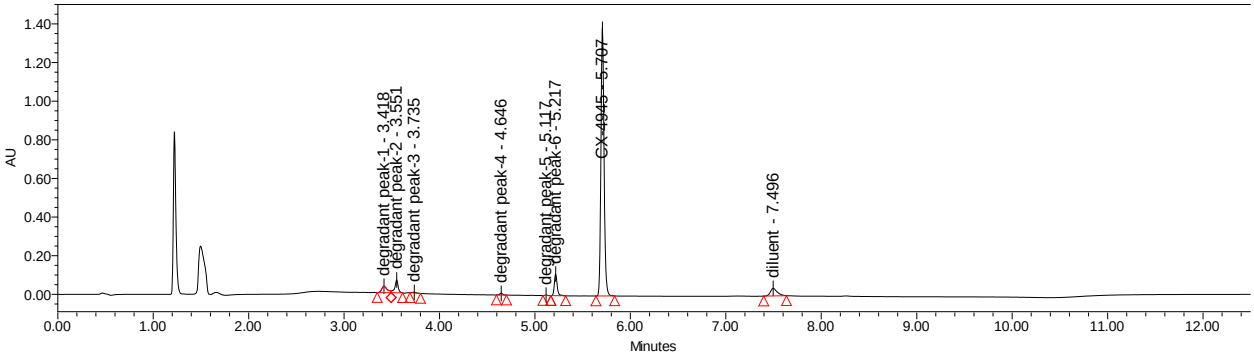


Figure 20. The plot of mean area response of CX-4945 vs. concentration – Assay level

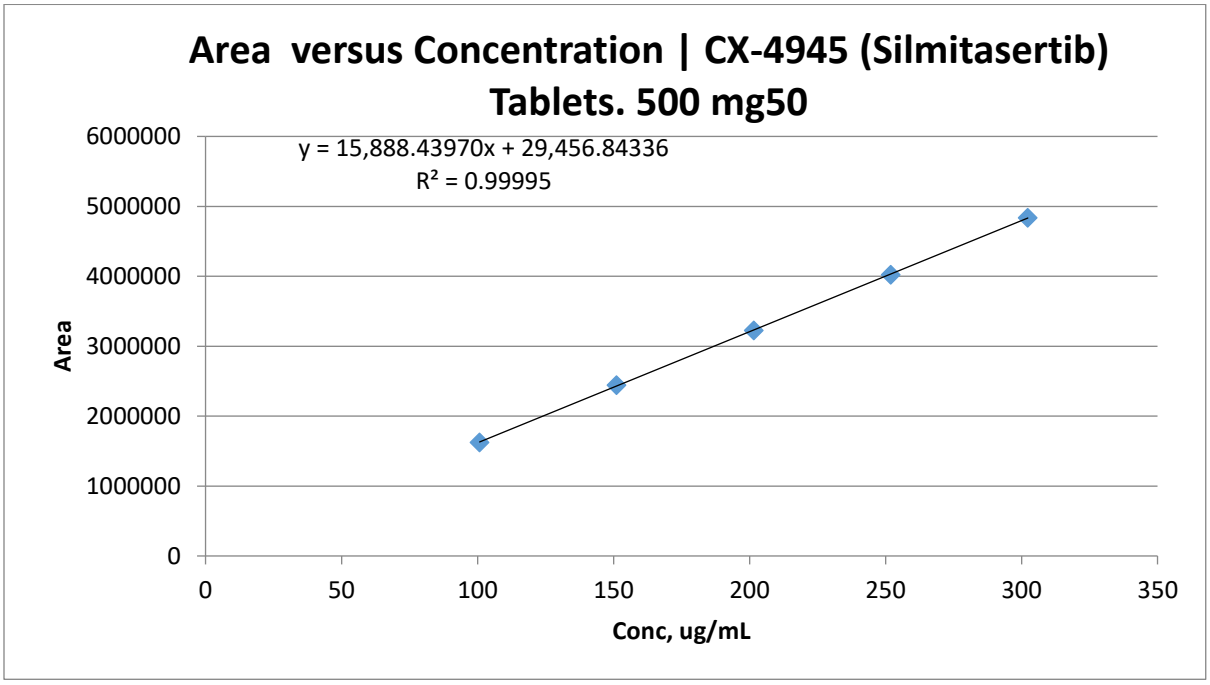


Figure 21. The plot of mean area response of CX-4945 vs. concentration – Impurity level

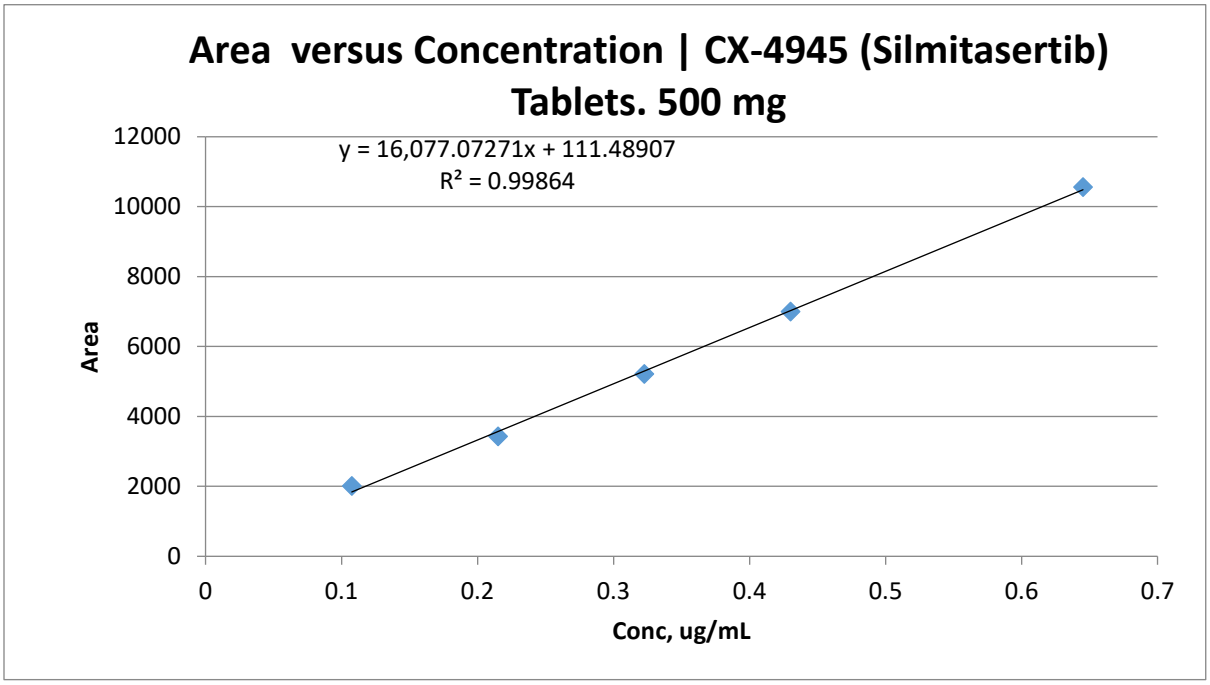
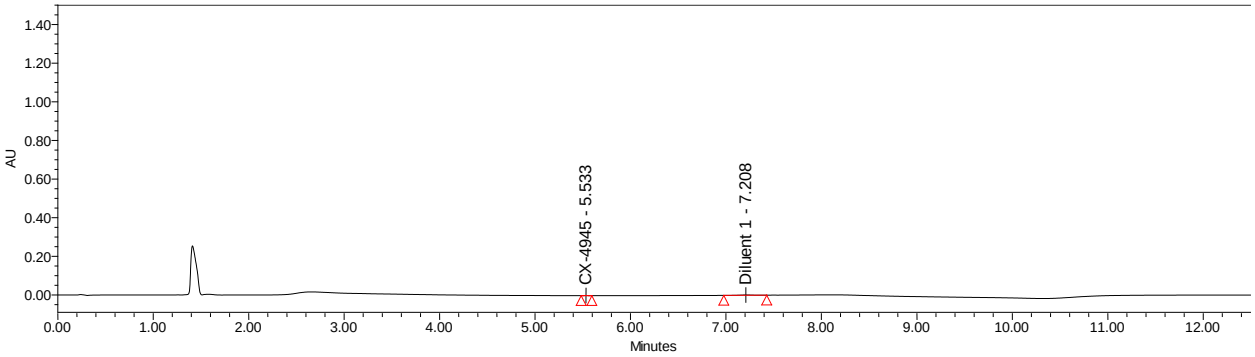


Figure 22. A representative chromatogram of the working QL solution.



16 CHANGES/DEVIATIONS AND INVESTIGATIONS

16.1 Changes to and Deviations from Protocol

Section in Protocol	Change
Table 2-1 (HPLC Parameters)	Typographical error in the column particle size. It should state 3.5 µm instead of 3µm.
Section 2.11.1 (Stock sample solution preparation)	The stock sample solution preparation for content uniformity was modified as follows: Accurately weigh 1 tablet and transfer into a 250-mL volumetric flask. Add about ¾ volume of diluent and swirl to avoid clumping. ***Mechanically shake for 15 minutes, sonicate for 30 minutes with occasional swirling, and then 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. This change in the stock sample preparation was inadvertently omitted in the protocol.
Section 9.5 (Acceptance Criteria) Precision and Intermediate Precision Study	The following requirements were missing in the acceptance criteria for the precision and intermediate precision study: <u>For Drug Product Assay</u>: • The RSD of the results from the Intermediate Precision study (n=6) is NMT 3.0%. • The absolute difference between the mean results obtained in the precision and intermediate precision studies is NMT 3.0%. <u>For Drug Product Content Uniformity</u>: • The absolute difference between the mean results obtained in the precision and intermediate precision studies is NMT 3.0%.
Section 10.2 (Filter study on assay sample solution)	Typographical error in the preparation of the working sample solution from the centrifuge sample solution. It should state "dilute 2.5 mL of the *supernatant* to 25 mL with diluent."

16.2 Investigations

None

Signature Manifest

Document Number: RPT MV 0188 **Revision:** 1

Title: CX-4945 (Silmitasertib) Tablets, 500 mg: Assay, RS, BU, CU and ID by RT Method Validation Report

All dates and times are in Eastern Time.

CX-4945 (Silmitasertib) Tablets, 500 mg: Assay, RS, BU, CU and ID by R...

Step 3 Customer Approval

Name/Signature	Title	Date	Meaning/Reason
Marjorie Cordero (MCORDERO)	Analytical Chemist - CMS	25 Jan 2022, 12:55:44 PM	Complete

P Step 4 Author Approval

Name/Signature	Title	Date	Meaning/Reason
Marjorie Cordero (MCORDERO)	Analytical Chemist - CMS	27 Jan 2022, 09:33:00 AM	Approved

P Step 4a Management Approval

Name/Signature	Title	Date	Meaning/Reason
Timothy Kim (TKIM)	Manager, ARD	25 Jan 2022, 01:47:36 PM	Approved
Shiying Tian (STIAN)	Director, AR&D	25 Jan 2022, 04:12:13 PM	Approved

P Step 4b QA Approval

Name/Signature	Title	Date	Meaning/Reason
Kirit Patel (KPATEL)	Director - QA	25 Jan 2022, 03:12:03 PM	Approved

Step 5 Set Effective Date

Name/Signature	Title	Date	Meaning/Reason
Marjorie Cordero (MCORDERO)	Analytical Chemist - CMS	27 Jan 2022, 12:05:35 PM	Approved