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| **Protocol Reference** | Method Validation Protocol PRO-02817-1 (Effective date: 11/09/23) | |
| **Notebook Reference** | ARD-0619  ARD-0640 | Pgs. 3-43  Pgs. 1-4 |
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# Introduction

This report summarizes the findings from the execution of method validation protocol PRO-02817 (v1.0), which pertains to the early phase method validation of the *Blend Assay,* *Blend Uniformity* and *Uniformity of Dosage Units* analytical procedures for TYRA-300 capsules (1 mg, 5 mg, and 10 mg).

Appropriate studies were performed in order to demonstrate that the proposed method is suitable for intended use. The corresponding protocol described the methodology for the validation of the analytical procedure and defines the criteria to assess the results.

The composition of the TYRA-300 tablets is summarized in **Table 1-1**. The three strengths are dose proportional.

Table 1-1: Ingredient Composition for TYRA-300 Tablets

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Ingredients** | **mg/unit** | | | **%w/w** | | |
| **1 mg** | **5 mg** | **10 mg** | **1mg** | **5mg** | **10mg** |
| TYRA-300-B01 salt | 1.282 | 6.41 | 12.82 | 6.41 | | |
| Lactose Monohydrate, NF (Fast Flo 316) – Part I | 1.784 | 8.92 | 17.84 | 8.92 | | |
| Lactose Monohydrate, NF (Fast Flo 316) – Part II | 3.568 | 17.84 | 35.68 | 17.84 | | |
| Lactose Monohydrate, NF (Fast Flo 316) – Part III | 3.568 | 17.84 | 35.68 | 17.84 | | |
| Microcrystalline Cellulose, NF (Avicel PH 102) | 9.00 | 45.00 | 90.00 | 45.00 | | |
| Croscarmellose Sodium NF (Ac-Di-Sol) | 0.4 | 2.00 | 4.00 | 2.00 | | |
| Colloidal Silicon Dioxide, NF (Cab-O-Sil) | 0.10 | 0.50 | 1.0 | 0.50 | | |
| Sodium Stearyl Fumarate, NF | 0.3 | 1.5 | 3.0 | 1.50 | | |
| **Core Mini-Tablets Total** | 20 | 100 | 200 | **100.00** | | |
| Opadry AMB II white 88A180040 | 2 | 10 | 20 | 10.00 | | |
| Purified Water | NA | N/A | NA | n/a | | |
| **Talc Blending** |  |  |  |  | | |
| Talc, USP | 0.04 | 0.20 | 0.40 | 0.20 | | |
| **Capsule Fill Weight** | 22 | 110 | 220 | **110.00** | | |

The method validation will be performed in accordance with Frontida’s Standard Operating Procedure for Validation of Analytical Methods, SOP-01377 (SOP MPC QC/RD-017) (current version), which is based on the ICH guidelines Q2(R1). The following characteristics/parameters were evaluated:

* System Suitability
* Specificity (Interference and Identification)
* Linearity and Range
* Accuracy by Spiked Recovery
* Precision
* Filtration Study
* Stability studies for the standard solution, sample solution, and mobile phases

# Analytical Procedure

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

## Chromatographic Parameters

Table 2-1: HPLC Parameters

|  |  |  |  |
| --- | --- | --- | --- |
| **Column** | Waters Cortecs, C18: 2.1 x 100 mm, 1.6 µm  PN: 186007095 | | |
| **Mobile Phase A** | 0.1% TFA in water | | |
| **Mobile Phase B** | 0.1% TFA in acetonitrile | | |
| **Needle Wash** | 90% methanol/ 10% water | | |
| **Purge/Seal Wash** | 20% methanol/ 80% water | | |
| **Needle Wash Time** | 15 seconds pre/30 seconds post | | |
| **Gradient Program** | **Time (min)** | **Mobile Phase A** | **Mobile Phase B** |
| 0 | 80 | 20 |
| 1.0 | 80 | 20 |
| 2.0 | 50 | 50 |
| 4.0 | 50 | 50 |
| 4.1 | 5 | 95 |
| 5.5 | 5 | 95 |
| 5.6 | 80 | 20 |
| 7.0 | 80 | 20 |
| **Detection** | 262 nm | | |
| **Detector Sampling Rate** | 10 pts/sec | | |
| **Flow Rate** | 0.4 mL/min | | |
| **Column Temperature** | 40°C ± 3°C | | |
| **Sample Compartment Temperature** | 5°C ± 4°C | | |
| **Injection Volume** | 3 μL | | |
| **Run Time** | 7 minutes | | |

## Reagents and Materials

Purified Water, Millipore

Acetonitrile, HPLC Grade

Methanol, HPLC Grade

Trifluoroacetic Acid (TFA), HPLC Grade

Tyra-300-B01, Reference Standard (RS)

Pall Acrodisc 0.2-µm PTFE 25mm syringe filter

## Mobile phase A (0.1% TFA in Water)

Combine 1.0 mL of trifluoroacetic acid with 1000 mL of purified water in a suitable container. Mix well and degas.

## Mobile phase B (0.1% TFA in 70% Acetonitrile)

Combine 1.0 mL of trifluoroacetic acid with 1000 mL of acetonitrile in a suitable container. Mix well and degas.

## Diluent Preparation

Prepare a mixture of methanol and purified water at a ratio of 90:10. Mix well.

## Standard Solution Preparation

Note—Protect standard solutions from light.

Prepare a check standard solution in a similar manner.

### Stock Standard Solution Preparation

Accurately weigh and quantitatively transfer about 65 mg of TYRA-300-B01 RS into a 100-mL volumetric flask. Add diluent to about 2/3 of flask volume and briefly sonicate (about 5 minutes) to dissolve the standard. Dilute to volume with diluent, mix well and label as the Stock standard solution.

The concentration of TYRA-300 free base is about 0.5 mg/mL.

### Working Standard Solution Preparation

Pipette 10.0 mL of stock standard solution into a 50-mL volumetric flask. Dilute to volume with diluent and mix well.

The concentration of TYRA-300 free base is about 0.1 mg/mL.

## Blend Assay Sample Solution Preparation

Note—Protect sample solutions from light.

Prepare the sample by weighing an equivalent of 10 capsules into the appropriate flask in order to achieve a free base concentration of TYRA-300 between 0.05 mg/mL and 0.15 mg/mL. Add diluent to about 2/3 of the flask volume to dissolve TYRA-300. Sonicate for 10 minutes. After equilibration to room temperature, dilute to volume with diluent and mix well. Filter a portion of sample through a 0.2-µm PTFE 25mm syringe filter, after discarding first 2 mL.

## Blend Uniformity Sample Solution Preparation

Note—Protect sample solutions from light.

Wipe the outsides of the sample bottles. Place the bottles upright and tap gently to dislodge any powder adhering to the liner of the caps. Gently remove the cap and weigh the bottle and sample (do not weigh the cap). Quantitatively transfer the entire bottle contents into a suitable volumetric flask as outlined in **Table 2-1**, rinsing the bottle a few times with the diluent to effect complete transfer.

**Table 2-1: Sample Preparation for Blend Uniformity**

|  |  |  |  |
| --- | --- | --- | --- |
| **Dosage Strength** | **Run Weight** | **Sample Dosage (x = run weight)** | **Volumetric Flask**  **(mL)** |
| 1 mg | 22 mg | 1x | 10 |
| 2x | 20 |
| 3x | 20 |
| 5 mg | 110 mg | 1x | 50 |
| 2x | 100 |
| 3x | 100 |
| 10 mg | 220 mg | 1x | 100 |
| 2x | 200 |
| 3x | 250 |

Add diluent to about 2/3 of the flask volume to dissolve TYRA-300. Sonicate for 10 minutes. After equilibration to room temperature, dilute to volume with diluent and mix well. Filter a portion of sample through a 0.2-µm PTFE 25mm syringe filter, after discarding first 2 mL.

Allow the sample bottles to dry and reweigh. Use this weight as the tare weight for the calculation. Determine the sample weight by subtracting the bottle tare weight from the weight of the bottle and samples as obtained above.

## Content Uniformity Solution Preparation

Note—Protect sample solutions from light.

Weigh ten capsules individually and record the weight (for information only). Open ten capsules and transfer contents to individual volumetric flasks according to the table below and record the empty shell weight (for information only).

Table 2-2: Content Uniformity Sample Preparation

|  |  |  |
| --- | --- | --- |
| **Dosage Strength** | **Volumetric Flask  (mL)** | **TYRA-300 Concentration (mg/mL)** |
| 1 mg | 10 | 0.1 mg/mL |
| 5 mg | 50 | 0.1 mg/mL |
| 10 mg | 100 | 0.1 mg/mL |

Add *purified* *water* to 10% of flask volume and briefly sonicate to disperse coating (about 2 minutes). Fill with *methanol* to about 2/3 of flask volume and sonicate 15 minutes and shake 15 minutes. After equilibration to room temperature, dilute flask to volume with methanol and mix well. Centrifuge portion of sample at 12000 rpm for 10 minutes and transfer the supernatant to an HPLC vial for analysis.

Alternatively, filter a portion of sample through a 0.2-µm PTFE 25 mm syringe filter, after discarding first 2 mL.

## Procedure

Separately inject equal volumes (3 µL) of the diluent, working standard and check standard, and sample solution. Record the chromatograms and measure the peak area responses of the TYRA-300 peak.

**Example of Injection Sequence**

|  |  |
| --- | --- |
| Solutions | Number of Injections |
| Diluent | ≥1 |
| Working Standard Solution | 5 |
| Check Standard Solution | 1 |
| Procedural Control Standard (PCS) | 1 |
| Sample Solution | ≤12 |
| Procedural Control Standard (PCS) | 1 |

## System Suitability Requirements

* The diluent blank injection should have no peaks which significantly interfere (NMT 0.5%) with the quantitation of TYRA-300.
* The RSD of the TYRA-300 peak area responses for the five (5) consecutive injections of working standard solution is NMT 2.0%.
* The percent recovery of TYRA-300 in the check standard solution is within 98.0% - 102.0%.
* The percent deviation between average of 5 consecutive working standard and each bracketing standard injection must be NMT 3.0%.

## Calculations

Calculate the % Label Claim as follows:

For Blend Uniformity:

For Content Uniformity:

Where,

|  |  |  |
| --- | --- | --- |
| Ru | : | The area response of TYRA-300 in the sample solution |
| Rs | : | The area response of TYRA-300 in the standard solution |
| Ws | : | Weight of the TYRA-300 standard, in mg |
| P | : | Purity of standard expressed as % Purity/100% |
| C | : | Free base conversion factor, 0.7796 |
| VF | : | Volumetric flask used for sample solution, in mL |
| WSpl | : | Weight of the TYRA-300 sample, in mg |
| RW | : | Run Weight, in mg |
| LC | : | Label claim, in mg |

**Note** – C may already be included in calculation for Purity. If so, then C should be omitted from above calculation.

For Content Uniformity, calculate the Acceptance Value as follows:

Acceptance Value = |M - X̄| + ks

Where,

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

# Instruments and Equipment

* Waters Acquity UPLC, HClass equipped with PDA/UVA detector
* UPLC Instrument: ARDLC09, Cal Due: 03/24
* Column: Cortecs C-18, 100 x 2.1 mm, 1.6 µm, S/N 01623311118584

# Reagents and MAterials

Reagents:

* Purified water, Millipore, In-House, Cal Due: 02/24
* Methanol, HPLC Grade, Supelco, Lot# 63117, exp: 11/26
* Acetonitrile, HPLC Grade, Omnisolv, Lot# 63224, exp: 09/24
* Trifluoroacetic acid, HPLC Grade, Fisher, Lot# 214204, exp: 01/25

Materials:

* TYRA-300-B01 Reference Standard, Cambrex, Lot# 006BJF062, exp: 10/24, purity = 76.09%
* TYRA-300 Sprinkle Capsule 1 mg, Lot# NB1806:75
* TYRA-300 Sprinkle Capsule 5 mg, Lot# NB1806:73
* TYRA-300 Sprinkle Capsule 10 mg, Lot# NB1806:71
* 0.2 µm PTFE 25 mm syringe filter, Pall, Lot# 7273734A

# System Suitability

The system suitability was successfully demonstrated for HPLC analysis. Representative results are summarized below in **Table 5-1**.

**Table 5-1: Representative System Suitability Results**

|  |  |
| --- | --- |
| **System Suitability Criteria** | **Result** |
| Diluent Interference | Not Detected |
| Standard % RSD (n=5) | 0.5% |
| Check Std % Recovery | 99.8% |
| Bracketing Standard % Deviation | 0.1%-0.2% |
| Acceptance Criteria:   * The diluent blank injection should have no peaks which significantly interfere (NMT 0.5%) with the quantitation of TYRA-300. * The RSD of the TYRA-300 peak area responses for the five (5) consecutive injections of working standard solution is NMT 2.0%. * The percent recovery of TYRA-300 in the check standard solution is within 98.0% - 102.0%. * The percent deviation between average of 5 consecutive working standard and each bracketing standard injection must be NMT 2.0%. | |

Reference: ARD-0619/20

# SPECIFICITY STUDY (INTERFERENCE AND IDENTIFICATION)

Specificity studies were performed in order to determine peak identities as well as evaluate whether there are any significantly interfering peaks arising from the diluent or placebo that may affect the quantitation of the intended analytes.

## Diluent Interference Solution Preparation

Used diluent as the diluent interference solution.

## Placebo Solution Preparation

Accurately weighed 210 mg placebo powder and quantitatively transferred into a 100 mL volumetric flask. Added 2 mL of purified water and sonicated 5 minutes. Filled with methanol to about 2/3 of flask volume and sonicated 30 minutes and shook 30 minutes. After equilibration to room temperature, diluted flask to volume with methanol and mixed well. Centrifuged portion of sample at 12000 rpm for 10 minutes and transferred the supernatant to an HPLC vial for analysis.

## Results and Discussion

Results for specificity are summarized below in **Table 6-1**. All acceptance criteria were met.

**Table 6-1: Specificity Results**

|  |  |
| --- | --- |
| **Interference at Retention Time of TYRA-300** | **Result** |
| Dissolution Medium | Not Detected |
| Placebo | Not Detected |
| Acceptance Criteria:   * The diluent and placebo solutions do not show any significantly interfering peaks near the retention time of TYRA-300 (NMT 0.5%). | |

Reference: ARD-0619/30

**Figure 1** is a representative chromatogram of the diluent solution.

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

**Figure 3** is a representative chromatogram of the working standard solution.

# Linearity

Linearity of TYRA-300 was evaluated from a concentration of 0.05 mg/mL to 0.15 mg/mL, which corresponds to 50% to 150%, respectively, of the nominal TYRA-300 concentration in the standard and sample solutions.

## Stock TYRA-300 Linearity Solution Preparation

Accurately weighed and quantitatively transferred about 65 mg of TYRA-300-B01 RS into a 100-mL volumetric flask. Added diluent to about 2/3 of flask volume and briefly sonicated (about 5 minutes) to dissolve the standard. After equilibration to room temperature, diluted to volume with diluent and mixed well.

The concentration of TYRA-300 free base is about 0.5 mg/mL.

## Working TYRA-300 Linearity Solutions Preparation

Prepared the working linearity solutions for the L1 to L5 levels as directed in **Table 7‑1**. Diluted each to volume with the diluent and mixed well.

Table 7-1: Preparation of working TYRA-300 linearity solutions

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Assay Linearity Level | Nominal Conc.  (%) | Volume of Stock TYRA-300 Linearity Solution (mL) | Flask  Volume  (mL) | Approx. Conc. Of TYRA-300  (mg/mL) |
| L1 | 50 | 2.5 | 25 | 0.05 |
| L2 | 80 | 4.0 | 25 | 0.08 |
| L3 | 100 | 10.0 | 50 | 0.10 |
| L4 | 120 | 6.0 | 25 | 0.12 |
| L5 | 150 | 7.5 | 25 | 0.15 |

## Results and Discussion

Results from linearity study are summarized in **Table 7-2**. All criteria were met. Linearity plot is included as **Figure 4**.

**Table 7-2: Linearity Results**

|  |  |  |  |
| --- | --- | --- | --- |
| **Level** | **Level (%)** | **Conc. TYRA-300 Free Base (mg/mL)** | **Peak Area** |
| L1 | 50 | 0.0495 | 744691 |
| L2 | 80 | 0.0793 | 1182974 |
| L3 | 100 | 0.0991 | 1476548 |
| L4 | 120 | 0.1190 | 1768196 |
| L5 | 150 | 0.1487 | 2213580 |
| Y-Intercept | | | 9114 |
| % Intercept (Relative to L3) | | | 0.6% |
| Correlation Coefficient, r | | | 1.000 |
| **Acceptance Criteria:**   * Meet the linearity range of a minimum of five consecutive levels. * The correlation coefficient, r, is NLT 0.999. * The y-intercept relative to the 100% nominal level is NMT 2%. | | | |

Reference: ARD-0619/41-42

# Accuracy by Spiked recovery

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

The accuracy study was performed by adding known amounts of TYRA-300 onto a corresponding amount of placebo powder. The accuracy was evaluated from a TYRA-300 concentration of 0.05 mg/mL to 0.15 mg/mL, which corresponds to 50% to 150% of the nominal sample solution concentration.

## Working Spiking Solution Preparation

Used *Stock Standard* solution (**Section 2.6.1**).

The concentration of TYRA-300 free base was about 0.5 mg/mL.

## Recovery Sample Solution Preparation

Accurately weighed and quantitatively transferred portions of TYRA-300 placebo powder into volumetric flasks as shown in **Table 8-1**. Added volumes of the *Working Spiking* solution as shown in **Table 8-1**. Filled with *diluent* to about 2/3 of flask volume and sonicated 15 minutes and shook 15 minutes. After equilibration to room temperature, diluted flask to volume with diluent and mixed well. Filtered a portion of sample through a 0.2µm PTFE 25mm syringe filter, after discarding first 2 mL.

Prepared each level in triplicate.

**Table 8-1: Preparation of the recovery sample solutions**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Recovery Level | Nominal Conc.  (%) | Volume of Working Spike Solution (mL) | Weight of Placebo Powder  (mg) | Volumetric Flask  (mL) | Approx. TYRA-300 Conc.  (mg/mL) |
| R1 | 50 | 5.0 | 105 | 50 | 0.05 |
| R2 | 100 | 10.0 | 105 | 50 | 0.10 |
| R3 | 150 | 15.0 | 105 | 50 | 0.15 |

## Control Sample Preparation

Accurately weighed 105 mg placebo powder and quantitatively transferred into a 50 mL volumetric flask. Added 5 mL of purified water and sonicated 5 minutes. Filled with methanol to about 2/3 of flask volume and sonicated 30 minutes and shook 30 minutes. After equilibration to room temperature, diluted flask to volume with methanol and mixed well.

Filtered a portion of sample through a Pall Acrodisc, 0.2-µm PTFE 25 mm syringe filter, after discarding first 2 mL.

## Results and Discussion

All criteria were met. Results from recovery study are summarized in **Table 8-2**:

**Table 8-2: Recovery Results**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Level** | **Sample** | **Peak Area** | **% Recovery** | **Average % Recovery** | **% RSD** |
| R1 | 1 | 724979 | 98.51 | 100 | 1 |
| 2 | 739558 | 100.49 |
| 3 | 737885 | 100.27 |
| R2 | 1 | 1463098 | 99.30 | 100 | 1 |
| 2 | 1478742 | 100.37 |
| 3 | 1463662 | 99.34 |
| R3 | 1 | 2187907 | 98.97 | 100 | 1 |
| 2 | 2209903 | 99.96 |
| 3 | 2209202 | 99.93 |
| **Acceptance Criteria:**   * The mean percent recovery is within 95-105%. * The % RSD of the triplicate preparations is NMT 3%. | | | | | |

Reference: ARD-0619/40

# Precision

## Precision

Prepared ten (10) sample solutions as directed in **Section 2.9** using TYRA-300 sprinkle capsules, 5 mg strength.

## Results and Discussion

All criteria were met. Results for precision study are summarized in **Table 9-1**:

**Table 9-1: Content Uniformity for NB1806:73, 5 mg**

|  |  |
| --- | --- |
| **Sample** | **% LC** |
| 1 | 105.00 |
| 2 | 107.64 |
| 3 | 106.82 |
| 4 | 106.44 |
| 5 | 106.27 |
| 6 | 105.86 |
| 7 | 99.11 |
| 8 | 104.71 |
| 9 | 105.43 |
| 10 | 105.16 |
| **Mean** | **105.2** |
| **Min** | **99.1** |
| **Max** | **107.6** |
| **AV** | **9.3** |
| **Acceptance Criteria:**   * The acceptance value (AV) is NMT 15.0. | |

Reference: ARD-0619/21

To calculate the acceptance value (AV), the following parameters were used:

|  |  |  |
| --- | --- | --- |
| k | : | 2.4 (for sample size of 10 units) |
| s | : | 2.3 (standard deviation) |
| M | : | 101.5 (mean ≥ 101.5%) |

# Filter Study

A filter study was performed to evaluate the suitability of the filters used for the sample solution preparation.

## Filter Study on Diluent

Filtered a portion of the diluent through a Pall Acrodisc 0.2-µm PTFE filter and collected the first 2 mL of filtrate for each.

## Filter Study on Sample Solution

Filtered Sample:

Filtered a portion of the content uniformity sample solution prepared as per **Section 2.9** (Note**—**A sample solution prepared for **Section 6.1** was used) through a Pall Acrodisc 0.2-µm PTFE membrane and collected each aliquot portion as shown in **Table 10-1**.

Centrifuged Sample:

Additionally, centrifuged a portion of the same sample at 12000 rpm for 10 minutes.

Table 10-1: Collection of filtrate aliquots for filter study

|  |  |  |
| --- | --- | --- |
| **Aliquot** | **Filtration Fraction (mL)** | **Volume Collected (mL)** |
| 1 | 0 - 2 | 2 |
| 2 | 2 - 4 | 2 |
| 3 | 4 - 6 | 2 |

## Results and Discussion

All criteria were met. Results from filter study are summarized in **Table 10-2**. Discarding the first 2 mL before collecting filtrate aliquots is adequate.

Table 10-2: Filter Study Results

|  |  |  |
| --- | --- | --- |
| **Sample** | **Peak Area** | **% Relative Recovery** |
| Centrifuged | 1552434 | — |
| Filter 0-2 mL | 1542689 | 99.4 |
| Filter 2-4 mL | 1548124 | 99.7 |
| Filter 4-6 mL | 1552585 | 100.0 |
| **Acceptance Criteria:**   * No interference more than 2% is observed in the filtered diluent solution. * The relative recovery of TYRA-300 from the filtrate aliquots of the sample solution (calculated against the centrifuged sample solution) is within 98.0-102.0%. | | |

Reference: ARD-0619/39

# Stability Study

The stability of the standard and sample solutions were evaluated at normal laboratory environmental (NLEC) and refrigerated conditions (2-8°C) to determine whether they are stable for use within the set time frame at the storage condition.

The stability of the standard solution was determined by periodically evaluating the recovery of TYRA-300 in the solution against freshly prepared standard solutions.

The stability of the sample solution was determined by periodically quantitating the percent of TYRA-300 in the solution against freshly prepared standard solutions.

The stability of the mobile phase was evaluated concomitantly with that of the standard and sample solutions.

## Results and Discussion

For Standard Solution:

Stability results are summarized in **Table 11-1**. The acceptance criteria were not met for solutions stored at NLEC at the evaluated intervals. The acceptance criteria were met for the solutions stored at refrigerated conditions for all evaluated conditions.

Table 11-1: Standard Solution Stability Results

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Storage** | **Day** | **% Recovery** | **% Relative Recovery** | **Conclusion** |
| — | Initial | 99.76 | — | — |
| NLEC | 4 | 96.23 | 96.5 | Fail |
| 5 | 92.91 | 93.1 | Fail |
| Refrigerated | 4 | 98.69 | 98.9 | Pass |
| 5 | 98.26 | 98.5 | Pass |
| **Acceptance Criteria:**   * The standard solution is considered stable if the relative recovery of the solution that is tested for stability at the evaluated time interval is within 98.0-102.0% of the original results (t0). | | | | |

Reference: ARD-0619/43

For Sample Solution:

Stability results are summarized in **Table 11-2**. The acceptance criteria were not met for solutions stored at NLEC at the evaluated intervals. The acceptance criteria were met for the solutions stored at refrigerated conditions for all evaluated conditions.

Table 11-2: Sample Solution Stability Results

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Storage** | **Day** | **% Assay** | **% Relative Recovery** | **Conclusion** |
| — | Initial | 105.0 | — | — |
| NLEC | 4 | 98.42 | 94 | Fail |
| 5 | 93.65 | 89 | Fail |
| Refrigerated | 4 | 106.95 | 102 | Pass |
| 5 | 105.96 | 101 | Pass |
| **Acceptance Criteria:**   * The sample solution is considered stable if the relative recovery obtained at the evaluated time interval is within 100 ± 2% of the original results (t0). | | | | |

Reference: ARD-0619/43

For Mobile Phase:

Stability results are summarized in **Table 11-3**. Criteria were met for NLEC conditions for at least 5 days.

Table 11-3: Mobile Phase Stability Results

|  |  |  |  |
| --- | --- | --- | --- |
| **Day** | **System Suit Average Retention Time (min)** | **10% Range** | **Conclusion** |
| Initial | 3.4049 | 3.0644-3.7453 | — |
| 1 | 3.4229 | Pass |
| 4 | 3.4168 | Pass |
| 5 | 3.4159 | Pass |
| **Acceptance Criteria:**   * The mobile phase is considered stable if the mean of retention times of the standards in the system suitability is within 10% of that obtained from the initial run (t0). | | | |

Reference: ARD-0619/38

# Method Range

Method range is 0.05 mg/mL to 0.15 mg/mL for TYRA-300 (free base) based on successfully demonstrated linearity and accuracy/precision studies. This range corresponds to 50% to 150% of the nominal sample solution concentration (0.1 mg/mL).

# Conclusions

The method validation protocol PRO-02817 (v1.0) for TYRA-300 Sprinkle Capsules was successfully executed. The findings from the studies are provided below:

* **Specificity (Interference)**: Specificity (Interference) of the method was demonstrated. There were no peaks in diluent or placebo solutions at the retention time of the TYRA-300 peak.
* **Linearity**: Linearity of TYRA-300 (free base) was demonstrated for concentration range from 0.05 mg/mL to 0.15 mg/mL. This range corresponds to 50% to 150% of the nominal sample solution concentration (0.1 mg/mL).
* **Accuracy**: Accuracy of this method was demonstrated for concentration range from 0.05 mg/mL to 0.15 mg/mL for TYRA-300 (free base). This range corresponds to 50% to 150% of the nominal sample solution concentration (0.1 mg/mL).
* **Precision:** Precision of this method was demonstrated.
* **Standard Solution Stability**: The standard solution was found to be stable for at least 5 days stored at refrigerated conditions (2-8°C).
* **Sample Solution Stability**: The sample solution was found to be stable for at least 5 days stored at refrigerated conditions (2-8°C).
* **Mobile Phase Stability**: The mobile phase is stable for at least 5 days stored at ambient conditions.

# Figures

**Figure 1: Representative Chromatogram of the Diluent**

A graph of a person

Description automatically generated with medium confidence

**Figure 2: Representative Chromatogram of the Placebo**

A graph showing the size of a number

Description automatically generated

**Figure 3: Representative Chromatogram of the Working Standard**

A graph with a red line

Description automatically generated

**Figure 4: Plot of Area vs. Concentration of TYRA-300**

**Figure 5: Representative Chromatogram of the Recovery Sample Solution (100% Level)**

A graph of a red line

Description automatically generated

**Figure 6: Representative Chromatogram of the Precision Sample Solution**

A graph with lines and numbers

Description automatically generated

# Changes/Deviations

## Changes to and Deviations from Protocol

|  |  |
| --- | --- |
| **Section in Protocol** | **Changes/Deviation** |
| **Section 1, Table 1-1** | Corrected listed Croscarmellose Sodium NF (Ac-Di-Sol) mg/unit for 10 mg. |
| **Sections 2.6, 2.7, 2.8 & 2.9** | Added notes to protect solutions from light. These statements were added based on findings observed during standard solution stability testing during the execution of Dissolution Method Validation protocol PRO-02815**.** |
| **2.11 System Suitability Requirements** | Revised percent deviation between average of 5 consecutive working standards and each bracketing standard injection from 2.0% to 3.0% to align with SOP requirements |
| **7.2 Filter Study on Sample Solution** | Titan 0.45-µm PTFE membrane filter was not evaluated as part of the filter study. |