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| **Notebook Reference** | ARD-0639 pg. 1-68 |
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Table of contents

[1 Introduction 5](#_Toc152767802)

[2 Analytical Procedure 6](#_Toc152767803)

[2.1 Chromatographic Parameters 6](#_Toc152767804)

[2.2 Dissolution Conditions 6](#_Toc152767805)

[2.3 Reagents and Materials 6](#_Toc152767806)

[2.4 Mobile Phase Preparation (Water: ACN: TFA, 60:40:0.1) 7](#_Toc152767807)

[2.5 0.01 N Hydrochloric Acid Solution preparation 7](#_Toc152767808)

[2.6 Diluent/Dissolution Medium Preparation (0.05% CTAB in 0.01N HCl) 7](#_Toc152767809)

[2.7 Standard Solution Preparation 7](#_Toc152767810)

[2.7.1 Stock Standard Solution Preparation 7](#_Toc152767811)

[2.7.2 Working Standard Solution Preparation 7](#_Toc152767812)

[2.8 Sample Solution Preparation 7](#_Toc152767813)

[2.9 Injection Procedure 8](#_Toc152767814)

[2.10 System Suitability 8](#_Toc152767815)

[2.11 Calculation 8](#_Toc152767816)

[3 Instruments and Equipment 10](#_Toc152767817)

[4 Reagents and MAterials 10](#_Toc152767818)

[5 System Suitability 10](#_Toc152767819)

[6 Specificity study 11](#_Toc152767820)

[6.1 Dissolution Medium Interference Solution Preparation 11](#_Toc152767821)

[6.2 Placebo Interference Solution Preparation 11](#_Toc152767822)

[6.3 Results and Discussion 11](#_Toc152767823)

[7 Linearity 12](#_Toc152767824)

[7.1 Working Linearity Solution Preparation 12](#_Toc152767825)

[7.2 Results and Discussion 12](#_Toc152767826)

[8 Accuracy by “Spiked” recovery study 12](#_Toc152767827)

[8.1 Spiking Solution Preparation 13](#_Toc152767828)

[8.2 Recovery Sample Solution Preparation 13](#_Toc152767829)

[8.3 Results and Discussion 13](#_Toc152767830)

[9 Precision Study 14](#_Toc152767831)

[9.1 Results and Discussion 14](#_Toc152767832)

[10 Filter Study 16](#_Toc152767833)

[10.1 Filter Study on Dissolution Medium 16](#_Toc152767834)

[10.2 Filter Study Sample Preparation 16](#_Toc152767835)

[10.3 Results and Discussion 16](#_Toc152767836)

[11 Solution Stability 17](#_Toc152767837)

[11.1 Sample Solution Preparation 17](#_Toc152767838)

[11.2 Results and Discussion 17](#_Toc152767839)

[12 Method Range 19](#_Toc152767840)

[13 Conclusions 20](#_Toc152767841)

[14 Figures 21](#_Toc152767842)

[15 Changes/Deviations 24](#_Toc152767843)

[15.1 Changes to and Deviations from Protocol 24](#_Toc152767844)

# Introduction

This report summarizes the findings for the execution of method validation protocol PRO-02815 (v1.0) which pertains to the early phase method validation of the *Dissolution by HPLC* analytical procedure for the TYRA-300 sprinkle capsules. The composition of the TYRA-300 sprinkle capsules is given below in Table 1-1.

Table 1-1: TYRA-300 Sprinkle Capsule Formulation

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Ingredients** | **mg/unit** | | | **%w/w** | | |
| **1 mg** | **5 mg** | **10 mg** | **1 mg** | **5 mg** | **10 mg** |
| 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** | | |

Appropriate validation studies were performed by the Adare/Frontida BioPharm ARD department in order to demonstrate that the method is suitable for the intended use. The following analytical parameters and procedures were performed:

• System Suitability  
• Specificity (Interference)  
• Linearity and Range  
• Accuracy  
• Precision   
• Filter Study  
• Solution Stability (Standard, Sample, Mobile Phase)

# 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** | Zorbax Eclipse XDB C-18, 150 x 4.6 mm, 3.5 µm,  P/N 963967-902 |
| **Mobile Phase** | Water: Acetonitrile (60:40) with 0.1% TFA |
| **Needle Wash** | 50:50 Acetonitrile: Purified Water |
| **Needle Wash Setting** | Normal |
| **Detection** | 325 nm |
| **Flow Rate** | 1.0 mL/min |
| **Injection Volume** | 20 μL |
| **Column Temperature** | 40°C ± 3°C |
| **Run Time** | 6 minutes |

## Dissolution Conditions

**Table 2-2: Dissolution Conditions**

|  |  |
| --- | --- |
| **Medium** | 0.05% CTAB in 0.01 N HCl |
| **Volume** | 900 mL |
| **Apparatus** | USP Type II (Paddles) |
| **Sinkers** | Japanese |
| **Speed** | 75 RPM (raise to 250 rpm after 60 min pull) |
| **Time** | For Profile: 15, 30, 45, 60 and 75 min |
| **Temperature** | 37°C ± 0.5°C |

**Table 2-3: Autosampler Parameters**

|  |  |
| --- | --- |
| **Flush Volume** | 5.0 mL |
| **Offset Volume** | 4.0 mL |
| **Sampling Rate** | 10.0 mL/min |
| **Collection Rate** | 10.0 mL/min |
| **Other Rates** | 10.0 mL/min |
| **Flush Times** | 2 |

## Reagents and Materials

* Purified Water
* Acetonitrile, HPLC grade or equivalent
* Methanol, HPLC grade or equivalent
* Trifluoroacetic Acid, HPLC grade or equivalent
* Concentrated HCl, Reagent Grade or better
* Cetyltrimethylammonium Bromide (CTAB), Reagent Grade or equivalent
* TYRA-300-B01 Reference Standard
* Porous UHMW polyethylene filter, 10 µm, QLA P/N FIL010-01
* 0.45 µm wwPTFE filter, Pall P/N 4932

## Mobile Phase Preparation (Water: ACN: TFA, 60:40:0.1)

For 1 L, combine 600 mL water, 400 mL acetonitrile, and 1 mL TFA. Mix well and degas.

## 0.01 N Hydrochloric Acid Solution preparation

Add 0.83 mL of concentrated Hydrochloric Acid per 1 L of water. Mix well and degas.

## Diluent/Dissolution Medium Preparation (0.05% CTAB in 0.01N HCl)

Add 0.5 g of CTAB per 1L of 0.01N HCl. Stir, heat, and/or sonicate until CTAB is in solution.

## Standard Solution Preparation

**NOTE – Protect standard solutions from light.**

Prepare a Check Standard Solution using a similar procedure.

### Stock Standard Solution Preparation

Accurately weigh and quantitatively transfer about 65 mg of TYRA-300-B01 standard into a 100-mL volumetric flask. Add methanol: water (90:10) to about 2/3 of flask volume and briefly sonicate (about 5 minutes) to dissolve the standard. After equilibration to room temperature, dilute to volume with methanol: water (90:10), 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 1.0 mL from the stock standard into a 100-mL volumetric flask. Dilute to volume with dissolution medium, mix well and label as the working standard solution.

The concentration of TYRA-300 free base is about 0.005 mg/mL.

## Sample Solution Preparation

**NOTE – Protect sample solutions from light.**

* Transfer 900 mL of the medium to six dissolution vessels, assemble the apparatus and equilibrate the dissolution medium to 37.0 ± 0.5 °C.
* Weigh 6 capsules individually and record the weights, corresponding to each respective vessel for information only. Secure each capsule into a sinker.
* When the media equilibrates to 37.0 ± 0.5 °C, drop sinker (with capsule) into vessel and start the method.
* At the specified time point(s), manually withdraw an aliquot (5 mL) with a syringe from midway between the surface of the dissolution medium and the top of the rotating paddle and not less than 1-cm away from the wall of the dissolution vessel.
* For manual pull: Filter samples using a 0.45-µm wwPTFE filters, discarding the first 3 mL. Retain the remaining filtrate for analysis.
* For autosampler: Autosampler probes should be equipped with 10-µm full flow filters. For Distek 4300 Automatic Sampler Flush Volume and Offset Volume settings should be properly adjusted to ensure transfer lines and filters are properly flushed before samples are delivered into HPLC vials.

## Injection Procedure

The following injection procedure is recommended. However, the injection sequence can be adjusted accordingly to the actual test situation.

**Table 2-4: The typical injection sequence is shown below**

|  |  |
| --- | --- |
| **Sample Type** | **Number of Injections** |
| Diluent (dissolution medium) | ≥1 |
| Working standard solution | 6 |
| Check standard solution | 1 |
| Bracketing standard (Working standard solution) | 1 |
| Sample Solution | 1 (bracket up to 12 sample injections with bracketing standard) \* |
| Bracketing standard (Working standard solution) | 1 |

**\***Make one injection of working standard solution after every 12 injections of sample solution and at the end of the run

## System Suitability

* The dissolution media injections should have no peaks that elute at RRT of the TYRA‑300 peak or which significantly interfere with the TYRA-300 peak (NMT 2% relative to the first injection of working standard).
* The relative standard deviation (%RSD) for the last six (6) consecutive injections of the working standard solution is NMT 2% for the TYRA-300 peak.
* The percent recovery of the check standard solution is within 97% - 103%.
* The retention time for the TYRA-300 peak of the bracketing standards is within 20% of the average for the last six (6) consecutive injections of the working standard solution.

## Calculation

|  |  |  |
| --- | --- | --- |
| Where: | | |
| Cstd | : | Concentration of standard (mg/mL in free base) |
| Wstd | : | Weight of TYRA-300-B01∙2HCl in standard solution (mg) |
| VF | : | Volumetric flask of standard solution (mL) |
| Purity | : | Purity of reference standard as %/100 |
| CF | : | Free base conversion factor, 0.7796 |
| Achk | : | Area of TYRA-300 peak for *check standard solution* |
| Astd | : | Average area of TYRA-300 peak for *standard solution* (includes bracketing standards |
| Cstd | : | Standard concentration for *standard solution* (mg/mL in free base) |
| Cchk | : | Concentration in the *check standard solution* (mg/mL in free base) |
| **Note** – CF may already be included in calculation for Purity. If so, then CF should be omitted from above calculation. | | |

Calculate the % dissolved as follows:

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

% Dissolved of TYRA-300 for dissolution release profile (no dissolution medium replacement)

• Calculate sample concentration at each time point

• Calculate the dissolution release profile

|  |  |  |
| --- | --- | --- |
| Where: | | |
| Asmp | : | Peak area in the sample solution |
| Astd | : | Average peak area of TYRA-300 in all standard and PCS injections |
| Cstd | : | Concentration of standard (mg/mL in free base) |
| Ci | : | Concentration of sample concentration at i time point (mg/mL in free base) |
| V | : | Initial volume of dissolution medium (mL) |
| LC | : | Label claim capsule strength (mg) |
| Vr | : | Volume of dissolution medium removed for each measurement, in mL |
| n | : | Number of the measurements (1, 2, 3......n) |

# Instruments and Equipment

* Waters Alliance HPLC system

HPLC Instrument: ARDLC98, Cal. due: 07/24

Column: Zorbax Eclipse XDB C-18, 150 x 4.6 mm, 3.5 µm, S/N USWA035979

* Distek Evolution 6100 Dissolution system

Dissolution Instrument: ARD-DS-02, Cal. Due.: 04/24

Dissolution Instrument: ARD-DS-04, Cal. Due.: 01/24

* Distek Evolution 4300 Dissolution autosampler

Autosampler Instrument: ARD-AS-04, Cal. due: 03/24

# Reagents and MAterials

Reagents:

* Purified water, Millipore, In-House
* Methanol, HPLC Grade, Supelco, Lot# 63117, Exp. Date: 11/26
* Acetonitrile, HPLC Grade, Supelco, Lot# 63244, Exp. Date: 10/26; Lot#63159, Exp. Date: 08/26
* Trifluoroacetic acid, HPLC Grade, Lot# 62273319, Exp. Date: 06/26
* Hydrochloric acid, Reagent Grade, Lot# 218780, Exp. Date: 04/27
* Cetyltrimethylammonium bromide (CTAB), 99+%, Lot# A0422507, exp: 09/25

Materials:

* TYRA-300-B01 Reference Standard, Cambrex, Lot# 006BJF062, Exp. Date: 10/24, purity: 76.09%
* TYRA-300 Sprinkle Capsule 1 mg, Lot# NB1806:75
* TYRA-300 Sprinkle Capsule 10 mg, Lot# NB1806:71
* 0.45 µm wwPTFE 25 mm syringe filter, Pall, Lot# 16183086A, P/N 4932

# System Suitability

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

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

|  |  |
| --- | --- |
| **Criteria** | **Result** |
| Dissolution Media Interference | Not Detected |
| % RSD of Replicate Standard Injections (n=6) | 0.2% |
| Check Standard % Recovery | 100% |
| Bracketing Standard Retention Time | 2%-4% |
| **Acceptance Criteria:**  • The dissolution media injections should have no peaks that elute at RRT of the TYRA-300 peak or which significantly interfere with the TYRA-300 peak (NMT 2% relative to the first injection of working standard).  • The relative standard deviation (%RSD) for the last six (6) consecutive injections of the working standard solution is NMT 2% for the TYRA-300 peak.  • The percent recovery of the check standard solution is within 97-103%.  • The retention time for the TYRA-300 peak of the bracketing standards is within 20% of the average for the last six (6) consecutive injections of the working standard solution. | |

Reference: ARD-0639/13

# Specificity study

## Dissolution Medium Interference Solution Preparation

Used the dissolution medium.

## Placebo Interference Solution Preparation

Accurately weighed and quantitatively transferred about 210 mg of TYRA-300 placebo into a vessel containing 900 mL of dissolution medium. Added one empty capsule into the vessel. Performed the dissolution according to **Section 2.2** and prepared the sample solution as instructed in **Section 2.8**.

## Results and Discussion

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

**Table 6-1: Specificity Results**

|  |  |
| --- | --- |
| **Interference at Retention Time of TYRA-300** | **Result** |
| Dissolution Medium | Not Detected |
| Placebo | Not Detected |
| Acceptance Criteria:   * The dissolution medium and placebo solutions do not show any significantly interfering peaks near the retention time of TYRA-300 (NMT 2%). | |

Reference: ARD-0639/39

**Figure 1** is a representative chromatogram of the dissolution medium solution.

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

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

# Linearity

The linearity was studied from about 10% to about 300% of the TYRA-300 concentration of the working standard solution, which corresponds to about 0.5 µg/mL to about 15.0 µg/mL.

## Working Linearity Solution Preparation

Prepared the working linearity solutions as directed in **Table 7-1**. Diluted each to volume with the dissolution medium and mixed well.

**Table 7-1: Preparation of Working Linearity Solutions**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample** | **Level (%)** | **Volume of Stock Std**  **(mL)** | **Flask (mL)** | **Approx. Conc. TYRA-300**  **(µg/mL)** |
| L1 | 10 | 10.0 mL of L3 | 100 | 0.5 |
| L2 | 50 | 1.0 | 200 | 2.5 |
| **L3** | **100** | **1.0** | **100** | **5.0** |
| L4 | 150 | 1.5 | 100 | 7.5 |
| L5 | 300 | 3.0 | 100 | 15.0 |

## Results and Discussion

The linearity results are summarized in **Table 7-2**. All acceptance criteria were met. Linearity plot is included as **Figure 4**.

**Table 7-2**: **Linearity Results**

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample** | **Level (%)** | **Conc. TYRA-300**  **Free Base (µg/mL)** | **Peak Area** |
| L1 | 10 | 0.50 | 15800 |
| L2 | 50 | 2.48 | 80002 |
| L3 | **100** | 4.96 | 160732 |
| L4 | 150 | 7.44 | 241100 |
| L5 | 300 | 14.88 | 484829 |
| Y-Intercept | | | -866 |
| % Intercept (Relative to L3) | | | -0.5% |
| 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 nominal 100% level is NMT 2%. | | | |

Reference: ARD-0639/37

# Accuracy by “Spiked” recovery study

Accuracy by spiked recovery was performed to demonstrate that the method can achieve acceptable recoveries.

The accuracy was performed by spiking TYRA-300 drug substance into an amount of composite placebo corresponding to the 10 mg dosage strength. The accuracy was evaluated from about 4.5% to 135% of the nominal concentration of TYRA-300 in the sample solution of the 10 mg capsule, which corresponds to about 0.5 µg/mL to 15 µg/mL. (Note—This range corresponds to a nominal sample concentration of 45% to 1350% for the 1 mg capsule and 9% to 270% for 5 mg capsule.)

## Spiking Solution Preparation

Accurately weighed and quantitatively transferred about 32.5 mg of TYRA-300-B01 material into a 100-mL volumetric flask. Added methanol: water (90:10) 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 methanol: water (90:10), mixed well and labelled as the spiking solution.

The concentration of TYRA-300 free base was about 0.25 mg/mL.

## Recovery Sample Solution Preparation

Accurately weighed and quantitatively transferred the amounts of placebo and volumes of the spiking solution into flasks as described in **Table 8-1**. Added about 2/3 volume of dissolution medium along with one empty capsule. Placed the flasks into a water bath at 37°C and shook for 30 minutes. Equilibrated the solutions to room temperature, diluted to volume with dissolution medium and mixed well. Filtered an aliquot of the solution through a 0.45-µm wwPTFE filter, discarding the first 2 mL.

Prepared each recovery level in triplicate.

**Table 8-1: Preparation of Recovery Sample Solutions**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Level** | **Nominal Concentration (%) Relative to the 10 mg Capsule** | **Volume of Spike Solution (mL)** | **Placebo (mg)** | **Flask (mL)** | **Approx. Conc. TYRA-300 (µg/mL)** |
| R1 | 4.5 | 0.5 | 52 | 250 | 0.5 |
| R2 | 9.0 | 1.0 | 52 | 250 | 1.0 |
| R3 | 45 | 5.0 | 52 | 250 | 5.0 |
| R4 | 135 | 15.0 | 52 | 250 | 15.0 |

## Results and Discussion

All acceptance criteria were met. The recovery study results are summarized in **Table 8-2**.

**Table 8-2: Recovery Results**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Level** | **Sample** | **Peak Area** | **% Recovery** | **Mean  % Recovery** | **% RSD** |
| R1 | 1 | 16231 | 101.26 | 101 | 0.4 |
| 2 | 16242 | 101.32 |
| 3 | 16114 | 100.53 |
| R2 | 1 | 32263 | 100.63 | 100 | 0.3 |
| 2 | 32094 | 100.10 |
| 3 | 32054 | 99.98 |
| R3 | 1 | 161385 | 100.68 | 101 | 0.2 |
| 2 | 161138 | 100.52 |
| 3 | 161693 | 100.87 |
| R4 | 1 | 487050 | 101.28 | 101 | 0.1 |
| 2 | 487693 | 101.41 |
| 3 | 487680 | 101.41 |
| **Acceptance Criteria**  • The mean percent recovery is within 90%-110%. • The percent RSD among the recoveries of different sample preparations within the same concentration level is NMT 5%. | | | | | |

Reference: ARD-0639/28

# Precision Study

For Precision, a six-capsule dissolution profile was performed on TYRA-300 Capsules 1 mg and 10 mg as per **Section 2**. The API-to-placebo ratio is dose proportional, so the 1 mg and 10 mg were considered the worst-case scenarios.

## Results and Discussion

All acceptance criteria were met. Precision results are given in **Table 9-1** and **Table 9-2**.

**Figures 5** and **6** are representative chromatograms of the 1 and 10 mg sample solutions, respectively.

**Table 9-1: Dissolution Profile for NB1806:75, 1 mg**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **% Dissolved** | | | | |
| **Time Points (min)** | **15** | **30** | **45** | **60** | **75** |
| Vessel 1 | 91 | 101 | 104 | 104 | 105 |
| Vessel 2 | 92 | 102 | 104 | 105 | 106 |
| Vessel 3 | 93 | 101 | 104 | 105 | 105 |
| Vessel 4 | 73 | 92 | 102 | 105 | 107 |
| Vessel 5 | 72 | 88 | 96 | 100 | 103 |
| Vessel 6 | 89 | 99 | 102 | 102 | 102 |
| **Mean** | **85** | **97** | **102** | **104** | **105** |
| **Min** | **72** | **88** | **96** | **100** | **102** |
| **Max** | **93** | **102** | **104** | **105** | **107** |
| **%RSD** | **11.6** | **5.9** | **3.1** | **2.1** | **1.7** |
| **Acceptance Criteria**  The acceptance criteria below will be evaluated only at time points 15 minutes and earlier:  • The %RSD (n=6) is NMT 20%.  The acceptance criteria below will be evaluated only at time points later than 15 minutes:  • The %RSD (n=6) is NMT 10%. | | | | | |

Reference: ARD-0639/22

**Table 9-2: Dissolution Profile for NB1806:71, 10 mg**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **% Dissolved** | | | | |
| **Time Points (min)** | **15** | **30** | **45** | **60** | **75** |
| Vessel 1 | 86 | 96 | 100 | 101 | 102 |
| Vessel 2 | 79 | 94 | 98 | 100 | 102 |
| Vessel 3 | 91 | 100 | 103 | 104 | 105 |
| Vessel 4 | 75 | 90 | 94 | 96 | 101 |
| Vessel 5 | 80 | 90 | 94 | 96 | 101 |
| Vessel 6 | 80 | 93 | 97 | 98 | 101 |
| **Mean** | **82** | **94** | **98** | **99** | **102** |
| **Min** | **75** | **90** | **94** | **96** | **101** |
| **Max** | **91** | **100** | **103** | **104** | **105** |
| **%RSD** | **6.9** | **4.2** | **3.5** | **3.1** | **1.5** |
| **Acceptance Criteria**  The acceptance criteria below will be evaluated only at time points 15 minutes and earlier:  • The %RSD (n=6) is NMT 20%.  The acceptance criteria below will be evaluated only at time points later than 15 minutes:  • The %RSD (n=6) is NMT 10%. | | | | | |

Reference: ARD-0639/23

# Filter Study

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

## Filter Study on Dissolution Medium

Separately filtered portions of the dissolution medium previously heated to about 37°C through a 0.45-µm wwPTFE filter and 10-µm filter and collected the first 2 mL of filtrate for each.

## Filter Study Sample Preparation

Filtered Sample:

For Manual Collection: Collected filtrate aliquots as per **Table 10-1** from a one‑capsule dissolution of the 1 mg and 10 mg capsules as per **Section 2**. (Note—The 75 min timepoint solutions prepared for Precision (1 mg and 10 mg) (prepared as per **Section 9.1**) were used.)

For Autosampler Collection: Collected aliquot from a one-capsule dissolution of the 1 mg and 10 mg capsules as per **Section 2**. (Note—The 75 min timepoint solutions prepared for Precision (1 mg and 10 mg) (prepared as per **Section 9.1**) were used.)

Centrifuge Sample:

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

Table 10-1: Filter Study by Manual Collection

|  |  |
| --- | --- |
| **Solution** | **Fraction of Filtrate Solution (mL)** |
| 0.45 µm wwPTFE filter, Pall P/N 4932 | 0-1 discarded |
| 1-3 |
| 3-5 |

## Results and Discussion

All acceptance criteria were met for all aliquots evaluated. Filter data is included below in **Table 10-2**.

The 10 µm full flow was found to be suitable for use with the autosampler. The 0.45‑µm wwPTFE filter was found to be suitable for manual filtration at all evaluated discard volumes. The filtrate discard volume of 3 mL as per the sample preparation procedure was found to be suitable.

**Table 10-2: Filter Study Results**

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample** | **Filter** | **Peak Area** | **% Relative Recovery** |
| Blank | 0.45 µm wwPTFE | ND | **—** |
| 10 µm full flow | ND | **—** |
| NB1806:75  Vessel 1  75 minutes | Centrifuge | 37721 | **—** |
| Autosampler (10 µm full flow) | 37645 | **100** |
| Manual 0-1 mL (0.45 µm wwPTFE) | 37506 | **99** |
| Manual 1-3 mL (0.45 µm wwPTFE) | 37715 | **100** |
| Manual 3-5 mL (0.45 µm wwPTFE) | 37644 | **100** |
| NB1806:71  Vessel 1  75 minutes | Centrifuge | 368527 | **—** |
| Autosampler (10 µm full flow) | 368555 | **100** |
| Manual 0-1 mL (0.45 µm wwPTFE) | 369252 | **100** |
| Manual 1-3 mL (0.45 µm wwPTFE) | 369420 | **100** |
| Manual 3-5 mL (0.45 µm wwPTFE) | 368547 | **100** |
| ND: Not Detected  **Acceptance Criteria**  **•** The percent relative recovery in the filter sample solution against the centrifuged solution is 97‑103%. **•** Determine volume of filtrate to be discarded before collecting samples that meet acceptance criteria for each level. | | | |

Reference: ARD-0639/14

# Solution Stability

The standard and sample solutions were evaluated at normal laboratory environmental condition (NLEC) to determine the appropriate time frame for use. Their stabilities were determined by periodically evaluating the solutions for change in TYRA-300 against freshly prepared solutions.

Additionally, stability of the mobile phase was concurrently evaluated.

## Sample Solution Preparation

Precision Samples (vessel 1, 75 minutes) (1 mg and 10 mg) were used to evaluate stability of the sample solutions.

## Results and Discussion

For Standard Solution:

The standard solution was evaluated while protected from light at the aforementioned storage condition.

Note—The initial evaluations of the standard and subsequent additional solution stability studies are summarized in **Section 15.1**.

The stability results of the standard solution are summarized in **Table 11-1**. All acceptance criteria were met at each evaluated timepoint.

The standard solution was found to be stable for at least 4 days when protected from light and stored at normal laboratory environmental conditions.

**Table 11-1: Standard Stability Results**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Interval** | **Peak Area** | **% Recovery** | **% Relative Recovery** | **Conclusion** |
| Initial | 173555 | 102.3 | — | — |
| Day 3 | 174022 | 101.9 | 99.6 | Pass |
| Day 4 | 173763 | 101.3 | 99.0 | Pass |
| **Acceptance Criteria**  • The standard solutions are considered stable if the relative recovery result at each time interval is within the range of 100.0% ± 3.0% of the original results. | | | | |

Reference: ARD-0639/67

For Sample Solution:

The stability results of the sample solutions are summarized in **Table 11-2**. All acceptance criteria were met at each evaluated timepoint.

The sample solution was found to be stable for at least 4 days stored at normal laboratory environmental conditions. Based on the findings from the standard solution stability, the recommended storage will be to protect from light stored at normal laboratory environmental condition.

**Table 11-2: Sample Solution Stability Results**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sample** | **Interval** | **Peak Area** | **% Recovery** | **% Relative Recovery** | **Conclusion** |
| NB1806:75 Vessel 1-75 min  1 mg | Initial | 38027 | 104.5 | — | — |
| Day 1 | 37675 | 104.4 | 100 | Pass |
| Day 4 | 37564 | 103.6 | 99 | Pass |
| NB1806:71 Vessel 1-75 min  10 mg | Initial | 367308 | 100.9 | — | — |
| Day 1 | 369046 | 102.3 | 101 | Pass |
| Day 4 | 367836 | 101.5 | 101 | Pass |
| **Acceptance Criteria**  • The sample solutions are considered stable if the relative recovery result at each time interval is within the range of 100% ± 3% of the original results. | | | | | |

Reference: ARD-0639/38

For Mobile Phase:

The stability results for the mobile phase are summarized in **Table 11-3**. All acceptance criteria were met at each evaluated interval. The mobile phase was found to generate consistent chromatography for at least 8 days when stored at normal laboratory environmental conditions.

**Table 11-3: Mobile Phase Stability Results**

|  |  |  |  |
| --- | --- | --- | --- |
| **Interval** | **Mean Retention Time TYRA-300 (min)** | **Acceptable Range (min)** | **Conclusion** |
| Initial | 3.3431 | 3.0087 – 3.6774 | — |
| Day 3 | 3.2010 | Pass |
| Day 4 | 3.1924 | Pass |
| Day 7 | 3.1615 | Pass |
| Day 8 | 3.1785 | Pass |
| **Acceptance Criteria**  • The mobile phase is considered stable if at each time interval the mean retention times of TYRA-300 from the system suitability standard injections are within 10% of retention times in the initial run (t0). | | | |

Reference: ARD-0639/67

# Method Range

Method range is 0.5 µg/mL to 15 µg/mL for TYRA-300 (free base) based on successfully demonstrated linearity and accuracy/precision studies. This range corresponds to 4.5% to 135% of the nominal sample solution concentration for 10 mg (11.1 µg/mL).

# Conclusions

The method validation protocol PRO-02815 (v1.0) for TYRA-300 Sprinkle Capsules was successfully executed and found to be suitable for its intended use. The findings from the studies are provided below:

* **System Suitability:** The system suitability of the method was successfully established.
* **Specificity (Interference)**: Specificity (Interference) of the method was demonstrated. There were no peaks in medium 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 0.5 µg/mL to 15 µg/mL. This range corresponds to 4.5% to 135% of the nominal sample solution concentration for the 10 mg dose (11.1 µg/mL), 9.0% to 270% of the nominal sample concentration for the 5 mg dose (5.56 µg/mL), and 45% to 1350% of the nominal sample concentration for the 1 mg dose (1.11 µg/mL).
* **Accuracy**: Accuracy of this method was demonstrated from concentration range of 0.5 µg/mL to 15 µg/mL for TYRA-300 (free base). This range corresponds to 4.5% to 135% of the nominal sample solution concentration for the 10 mg dose (11.1 µg/mL), 9.0% to 270% of the nominal sample concentration for the 5 mg dose (5.56 µg/mL), and 45% to 1350% of the nominal sample concentration for the 1 mg dose (1.11 µg/mL).
* **Precision**: The method was demonstrated to be precise.
* **Standard Solution Stability**: The stability of standard solution was established as 4 days when protected from light stored at normal laboratory environmental conditions.
* **Sample Solution Stability**: The stability of standard solution was established as 4 days when protected from light stored at normal laboratory environmental conditions.
* **Mobile Phase Stability**: The mobile phase was found to be stable for at least 8 days stored at normal laboratory environmental conditions.

# Figures

**Figure 1: Representative Chromatogram of the Dissolution Medium**

A graph with numbers and lines

Description automatically generated

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

A graph showing a number of numbers

Description automatically generated with medium confidence

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

A graph of a person with red lines

Description automatically generated with medium confidence

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

**Figure 5 Representative Chromatogram of the 1 mg Precision Sample Solution at 30 min**

A graph of a graph showing a number of numbers

Description automatically generated with medium confidence

**Figure 6: Representative Chromatogram of the 10 mg Precision Sample Solution at 30 min**

A graph of a graph showing a red line

Description automatically generated with medium confidence

# Changes/Deviations

## Changes to and Deviations from Protocol

| **Protocol Section** | **Change/Deviation** |
| --- | --- |
| **Section 1, Table 1-1** | Corrected listed Croscarmellose Sodium (Ac-Di-Sol) mg/unit for 10 mg. |
| **Section 2.7 & Section 2.7.1, Section 9** | Notes to protect solutions from light was added based on the findings from the solution stability study.  A summary of the initial stability of the standard solution stored at NLEC conditions and results are given below:   |  |  |  |  |  | | --- | --- | --- | --- | --- | | **Day** | **Peak Area** | **% Recovery** | **% Relative Recovery** | **Conclusion** | | Initial | 173178 | 100.4 | — | — | | 1 | 166889 | 97.8 | 97.4 | Pass | | 4 | 162424 | 94.7 | 94.3 | Fail | | **Acceptance Criteria**  • The standard solutions are considered stable if the relative recovery result at each time interval is within the range of 100.0% ± 3.0% of the original results. | | | | |   Reference: ARD-0639/38  The relative recovery was found to decrease quickly over time. This trend was not observed for the sample solution (Table 11-2). The slight difference in composition of the two solutions (standard solution contained about 1% methanol while the sample solution contained no methanol) was investigated as a potential cause. A second evaluation was performed which substituted the 90% methanol for 100% acetonitrile to prepare the stock standard solution. The stability results are given in the table below:   |  |  |  |  |  | | --- | --- | --- | --- | --- | | **Day** | **Peak Area** | **% Recovery** | **% Relative Recovery** | **Conclusion** | | Initial | 175693 | 99.7 | — | — | | 1 | 171982 | 96.6 | 96.8 | Fail | | **Acceptance Criteria**  • The standard solutions are considered stable if the relative recovery result at each time interval is within the range of 100.0% ± 3.0% of the original results. | | | | |   Reference: ARD-0639/52  The standard again was found to degrade quickly. A difference in solution storage was identified (standard solutions stored on benchtop; sample solution vial stored in HPLC injection compartment) and hence, degradation due to ambient light exposure was investigated as a potential cause. A third evaluation was performed using the original 90% methanol to dilute the stock standard but using low-actinic glassware for both stock and working standard solutions. The solution stability results are summarized in in **Table 11-1**. The standard solution was found to be stable when stored in this manner. Due to these findings, notes were added to **Section** **2.7** and **2.8** to protect standard and sample solutions from light. |
| **Section 9.1** | Error in acceptance criteria.  The original acceptance criteria of RSD at NMT 5% for precision was in error. As per SOP MPC QC-RD 017-6 the criteria should have been the evaluation of RSD at NMT 10% at the stated Q value. Since this method/product is intended for early phase validation, the Q time point has not yet been established. For method precision the results for the entire profile should be evaluated. Since high variability of product release is expected at the early timepoints (15 min), the criteria for RSD of NMT 20% is appropriate. The criteria at later timepoints should be RSD of NMT 10%. (This criteria is in alignment with FDA guidance on F2 calculations.)  The acceptance criteria should be as follows:  *The acceptance criteria below will be evaluated only at time points 15 minutes and earlier: • The %RSD (n=6) is NMT 20%.*  *The acceptance criteria below will be evaluated only at time points later than 15 minutes:*  *• The %RSD (n=6) is NMT 10%.* |