Sr. No. in Scope NABL / NON NABL

**Flow Chart for Analysis of Tricyclazole Content in Formulation Sample**

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| **Date of Analysis** |  |

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| **S. No.** | **Step** | **Execution** | | **Executed by** |
| **R1** | **R2** |
| 1. | Sample No. |  |  |  |
| 2. | Name of Sample |  |  |  |
| 3. | **Procedure** |  |  |  |
| **3.1.** | **Preparation of Mobile Phase** |  |  |  |
| 3.1.1 | Pipette out 10mL of 85% orthophosphoric acid (HPLC grade) in a 100 mL volumetric flask and make up to the mark with HPLC water. |  |  |  |
| 3.1.2. | Adjust the pH of HPLC water with 10% Ortho phosphoric acid (3.1.1) to pH 2.85 |  |  |  |
| 3.1.3. | Mix acetonitrile and pH adjusted water (3.1.2) in the proportion of 50:50 (v/v) |  |  |  |
| 3.1.4. | Pass through the 0.45 µm membrane filter under vaccum. |  |  |  |
| 3.1.5. | Sonicate the mixture to Homogenize. |  |  |  |
| 3.1.6 | Allow to attain room temperature. |  |  |  |
| **3.2** | **Preparation of Internal Standard Solution** |  |  |  |
| 3.2.1 | Weigh 1.5 g of Acetophenone in 100 ml volumetric flask | g | g |  |
| 3.2.2 | *Note down the serial No. of the balance log book* |  |  |  |
| 3.2.3 | Add 50 ml of Acetonitrile, mix & sonicate for 5 minutes. Cool to room temperature & make up to the mark with acetonitrile. |  |  |  |
| 3.2.4 | Stopper and shake well to homogenize. |  |  |  |
| **3.3** | **Preparation of standard solution** |  |  |  |
| 3.3.1 | Note the purity of the standard | % | % |  |
| 3.3.2 | Weigh 50 mg a. i. of Standard in a 100 ml volumetric flask | g | g |  |
| 3.3.3 | *Note down the serial No. of the balance log book* |  |  |  |
| 3.3.4 | Add 50 mL methanol, sonicate for 5 min to dissolve the material. |  |  |  |
| 3.3.5 | Add 5 ml of internal standard solution (3.2.4) | mL | mL |  |
| 3.3.6 | Dilute up to the mark with methanol and mix well (Stock A). |  |  |  |
| 3.3.7 | Pipette out 1 mL of Stock A (3.3.6) into a 100 mL volumetric flask |  |  |  |
| 3.3.8 | Dilute up to the mark with acetonitrile. Stopper and shake well to homogenize. |  |  |  |
| **3.4** | **Preparation of sample solution** |  |  |  |
| 3.4.1 | Note down the percent active ingredient declared on the sample | % | % |  |
| 3.4.2 | Weigh 50 mg a. i. of sample in a 100 ml volumetric flask | g | g |  |
| 3.4.3 | *Note down the serial No. of the balance log book* |  |  |  |
| 3.4.4 | Add 50 mL methanol, sonicate for 5 min to dissolve the material. | ml | ml |  |
| 3.4.5 | Add 5 ml of internal standard solution (3.2.4) |  |  |  |
| 3.4.6 | Dilute up to the mark with methanol and mix well (Stock B). |  |  |  |
| 3.4.7 | Pipette out 1 mL of Stock B (3.4.6) into a 100 mL volumetric flask |  |  |  |
| 3.4.8 | Dilute up to the mark with acetonitrile. Stopper and shake well to homogenize. |  |  |  |
| 3.4.9 | Filter the sample solution through 0.45µ membrane filter |  |  |  |
| **4.** | **HPLC Parameters** |  |  |  |
| **4.1** | **Column** |  |  |  |
| 4.1.1 | C18, Particle Size: 5µ |  |  |  |
| 4.1.2 | Length: 250 mm |  |  |  |
| 4.1.3 | I.D.: 4.6 mm |  |  |  |
| **4.2** | **Mobile Phase** |  |  |  |
| 4.2.1 | Acetonitrile : Water, pH adjusted to 2.85 (50:50) |  |  |  |
| 4.2.2 | Flow Rate : 0.85 ml/min |  |  |  |
| **4.3** | **Detector:** UV |  |  |  |
| **4.4** | **Wave Length**: 231 nm |  |  |  |
| **4.5** | **Injection Volume:** 20µl |  |  |  |
| 5. | **Result** |  |  |  |
| Sample chromatogram no. |  |  |  |
| Standard chromatogram no. |  |  |  |

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| **6. Calculation:**  Tricyclazole content, A2 x A3 x M1  % by mass = ------------------- x P  A1 x A4 x M2  **Where,**  M1 =Mass in ‘g’ of Tricyclazole standard  M2 =Mass in ‘g’ of sample taken for test  A1 = Peak area of Tricyclazole in the standard solution A2 = Peak area of Tricyclazole in the sample solution  A3 = Peak area of internal standard in the standard solution  A4 = Peak area of internal standard in the sample solution   P = Percent purity of Tricyclazole standard |
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**Result:**

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| **Sl.No.** | **Name of test** | **Result** | **Unit** | **Method of Analysis** |
| 1. | Active ingredient |  | % | Customer method |
| Remark / Reference : | | | | |

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| --- | --- | --- |
| Analyzed by | Name |  |
| Dated signature |  |
| Checked by | Name |  |
| Dated signature |  |