Sr. No. in Scope NABL / NON NABL

**Flow Chart for Analysis of Thiophanate methyl content in formulation sample**

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| --- | --- |
| **Date of Analysis**  |  |

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No.** | **Step** | **Execution** | **Executed by** |
| 1. | Sample No. | **R1** | **R2** |  |
| 2. | Name of Sample |  |  |  |
| 3. | **Procedure** |  |  |  |
| **3.1.** | **Preparation of Mobile Phase**  |  |  |  |
| 3.1.1. | Filter acetonitrile , methanol and water through 0.45 µ membrane filter under vacuum. |  |  |  |
| 3.1.2. | Mix acetonitrile methanol and water in the proportion of 1:1:2, *v/v* |  |  |  |
| 3.1.3. | Homogenize the mixture using the ultrabath sonicator. |  |  |  |
| 3.1.4. | Allow to attain room temperature. |  g | g |  |
| **3.2** | **Preparation of Internal Standard Solution** |  |  |  |
| 3.2.1 | Weigh 50 mg of Propyl 4- Hydroxy benzoate in a 100 mL volumetric flask. |  |  |  |
| 3.2.2 | *Note the serial No. of the balance log book* |  |  |  |
| 3.2.3 | Dissolve in about 30 mL of methanol and dilute up to the mark with methanol. |  |  |  |
| **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  |  mg | m g |  |
| 3.3.3 | *Note the serial No. of the balance log book* |  |  |  |
| 3.3.4 | Dissolve in 50 mL of methanol . | mL | mL |  |
| 3.3.5 | Dilute up to the mark with methanol (Stock A) |  |  |  |
| 3.3.6 | Pipette out 5 mL of Stock A (3.3.5) into a 50 mL volumetric flask |  |  |  |
| 3.3.7. | Add through pipette 5 mL of internal standard solution(3.2.3) |  |  |  |
| 3.3.8 | Dilute up to the mark with mobile phase (3.1.4) |  |  |  |
| **3.4** |  **Preparation of sample solution** |  |  |  |
| 3.4.1 | Note the percent active ingredient content declared on sample | % | % |  |
| 3.4.2 | Weigh 50 mg a. i. of Sample in a 100 ml volumetric flask | mg |  mg |  |
| 3.4.3 | *Note the serial No. of the balance log book* |  |  |  |
| 3.4.4 | Dissolve the sample in about 50 mL of methanol. | ml |  ml |  |
| 3.4.5 | Dilute up to the mark with methanol (Stock B) | % | % |  |
| 3.4.6 | Pipette out 5ml of Stock B (3.4.5) into a 50 ml volumetric flask | g | g |  |
| 3.4.7 | Add through pipette 5 mL of internal standard solution(3.2.3) |  |  |  |
| 3.4.8 | Dilute up to the mark with mobile phase (3.1.4) |  |  |  |
| 4. | **HPLC Parameters** |  |  |  |
| **4.1** | **Column** |  |  |  |
| 4.1.1 | C8, Particle Size: 10 µ  |  |  |  |
| 4.1.2 | Length: 250 mm |  |  |  |
| 4.1.3 | I.D.: 4.6 mm |  |  |  |
| **4.2** | **Mobile Phase** |  |  |  |
| 4.2.1 | Acetonitrile : methanol : water (1:1:2), v/v |  |  |  |
| 4.2.2 | Flow Rate : 1 ml/min |  |  |  |
| **4.3** |  **Detector:** UV |  |  |  |
| **4.4** |  **Wave Length**: 269 nm |  |  |  |
| **4.5** |  **Injection Volume:** 20µl |  |  |  |
| 5. | **Result** |  |  |  |
| Sample chromatogram no.  |  |  |  |
| Standard chromatogram no.  |  |  |  |

**6. Calculation:**

 A2 x A3 x M1

Thiophanate methyl content, % by mass = ------------------- x P

 A1 x A4 x M2

**R1**

**R2**

**Where,**

M1 =Mass in ‘g’ of Thiophanate methyl standard

 M2 =Mass in ‘g’ of sample taken for test

 A1 = Peak area of Thiophanate methyl in the standard solution

 A2 = Peak area of Thiophanate methyl 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 Thiophanate methyl standard

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| --- | --- | --- | --- | --- |
| **Sr. No.** | **Name of test** | **Result** | **Unit** | **Method of Analysis**  |
| 1. | **Active ingredient** |  | **%** | IS 14551-1998 (Reaffirmed 2009) |
| Remark / Reference  |
| Analyzed by | Name  |  |
| Dated signature |  |
| Checked by | Name  |  |
| Dated signature |  |