Sr. No. in Scope NABL / NON NABL

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

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

|  |  |  |  |
| --- | --- | --- | --- |
| **Sl. No.** | **Step** | **Execution** | **Executed By** |
| 1. | Sample No. |  |  |
| 2. | Name of Sample |
|  | Sample Description |
| 3. | **Procedure** |
| **3.1 Preparation of Mobile Phase** |  |  |
| 3.1.1 | Weight of 85% phosphoric acid taken in a 2L volumetric flask and made up with HPLC water |  |  |
| 3.1.2 | This solution is a 0.0425% phosphoric acid aqueous solution with pH of approximately 2.7 |  |  |
| 3.1.3 | Mix Acetonitrile and water(*p*H 2.7) in the proportion of 10:90 (v/v)  |   |  |
| 3.1.4 | Pass through membrane filter under vacuum |  |  |
| 3.1.5 | Homogenize the mixture using a magnetic stirrer |  |  |
| 3.1.6 | Allow the mixture to attain room temperature |  |  |
| **3.2 Preparation of Internal Standard** |  |  |
| 3.2.1 | Weight of Benzamide taken in 1000 ml volumetric flask | g |  |
| 3.2.2 | Add 200 ml of methanol |  |  |
| 3.2.3 | Dissolve Benzamide in methanol completely. |  |  |
| 3.2.4 | Dilute up to the mark with Methanol |   |  |
| **3.3 Preparation of Standard** |  |  |
| 3.3.1 | Weigh the standard accurately equivalent to 100 mg active content in 100 ml volumetric flask. | g |  |
| 3.3.2 | Note the purity of standard | % |  |
| 3.3.3 | Add to it 4 ml of Internal standard solution in the flask. (3.2.4) |  |  |
| 3.3.4 | Dilute up to the mark with water of pH 2.7 (3.1.2) |  |  |
| 3.3.5 | Dissolve ultrasonically for 5 min |  |  |
| 3.3.6 | Filter through 0.2um filter and inject standard solution. |   |  |
|  **3.4 Preparation of Sample** |  |  |
| 3.4.1 | Note the purity of sample | % |  |
| 3.4.2 | Weight the sample accurately equivalent to 100 mg active content in 100 ml volumetric flask | g |  |
| 3.4.3 | Add to it 4 ml of Internal standard solution in the flask. (3.2.4) |  |  |
| 3.4.4 | Dilute up to the mark with water of pH 2.7 (3.1.2) |  |  |
| 3.4.5 | Dissolve ultrasonically for 5 min |  |  |
| 3.4.6 | Filter through 0.2um filter and inject standard solution. |  |  |
| 4. | **HPLC Parameters** |  |  |
| **4.1 Column** |  |  |
| 4.1.1 | Stainless Steel Packed with Reverse phase C8 |  |  |
| 4.1.2 | Length: 150 mm |  |  |
| 4.1.3 | I.D.: 4.6 mm |  |  |
| **4.2 Mobile Phase** |  |  |
| 4.2.1 | Acetonitrile : Water (10 : 90) |  |  |
| 4.2.2 | Flow Rate: 1.5 ml/min |  |  |
| **4.3 Detector:** UV |  |  |
| **4.4** **Wave Length**: 254 nm |  |  |
| **4.5 Injection Volume:** 20 µl |  |  |
| 5. | **Result** |  |  |
| Sample chromatogram no.  |  |
| Standard chromatogram no.  |  |

**6. Calculation:**

|  |  |
| --- | --- |
|  A2 x A3 x M1  Methomyl content, = ------------------- x P% by mass A1 x A4 x M2 | **Where,**M1 =Mass in ‘g’ of Methomyl standardM2 =Mass in ‘g’ of sample taken for test A1 = Peak area of Methomyl in the standard solution A2 = Peak area of Methomyl in the sample solutionA3 = Peak area of internal standard in the standard solution A4 = Peak area of internal standard in the sample solution P = Percent purity of Methomyl in the standard |

**Result:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sl. No.** | **Name of test** | **Result** | **Unit** | **Method of Analysis**  |
| 1. | Active ingredient |  | % | 1S 15614-2006(Reaffirmed 2010)  |
| Remark / Reference : |

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