Sr. No. in Scope NABL/ NON NABL

**Flow chart for analysis of Fipronil content in formulation sample**

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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sl. No.** | **Step** | **Execution** | | **Executed By** |
| 1. | Sample No. | **R1** | **R** |  |
| 2. | Name of Sample |  |  |  |
| 3. | **Procedure** |  |  |  |
| **3.1** | **Preparation of Standard** |  |  |  |
| 3.1.1 | Purity of standard | g |  |  |
| 3.1.2 | Weigh 0.1 g of Fipronil reference standard into a 100 ml volumetric flask. | % |  |  |
| 3.1.3 | *Note down the S.No. of balance log book* |  |  |  |
| 3.1.4 | Dissolve and make up the volume to the mark with  acetonitrile (Stock A) |  |  |  |
| 3.1.5 | Pipette out 10 ml of stock A (3.1.5) into another  100 ml volumetric flask. |  |  |  |
| 3.1.6 | Make up to the mark with acetonitrile and  shake well to homogenize (stock B). |  |  |  |
| **3.2** | **Preparation of Sample** |  |  |  |
| 3.2.1 | Note down the percent active ingredient content declared on the sample. |  |  |  |
| 3.2.2 | Weigh 0.1 g a.i. of Fipronil sample into a 100 ml volumetric flask. | g |  |  |
| 3.2.3 | *Note down the S.No. of balance log book* |  |  |  |
| 3.2.4 | Dissolve and make up the volume to the mark with  acetonitrile (Stock C) |  |  |  |
| 3.2.5 | Pipette out 10 ml of stock C (3.2.4) into another 100 ml  volumetric flask. |  |  |  |
| 3.2.6 | Make up to the mark with acetonitrile and shake well to  homogenize (stock D). |  |  |  |
| 4. | **HPLC Parameters** |  |  |  |
| **4.1** | **Column** |  |  |  |
| 4.1.1 | RP C-18 |  |  |  |
| 4.1.2 | Length: 25 cm |  |  |  |
| 4.1.3 | I.D.: 4.6 mm |  |  |  |
| **4.2** | **Mobile Phase** |  |  |  |
| 4.2.1 | Acetonitrile : Water (70:30) v/v |  |  |  |
| 4.2.2 | Flow Rate: 1.0 ml/min |  |  |  |
| **4.3** | **Detector:** UV Detector |  |  |  |
| **4.4** | **Wave Length**: 280 nm |  |  |  |
| **4.5** | **Injection Volume:** 20 µl |  |  |  |
| 5. | **Result** |  |  |  |
| Sample chromatogram no. |  | |  |
| Standard chromatogram no. |  | |  |

**6. Calculation:**

|  |  |
| --- | --- |
| Fipronil content, % by mass =  A1 x M1  = ------------- x P  A2 x M2 | **Where,**  M1 =Mass in ‘g’ of Fipronil in the standard solution  M2 =Mass in ‘g’ of sample taken for test  A1 = Peak Area of Fipronil in the sample solution  A2 = Peak Area of Fipronil in standard solution  P = Percent purity of Fipronil standard |

**7. Result:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sl. No.** | **Name of test** | **Result** | **Unit** | **Method of Analysis** |
|  |  |  |  | **Validated method by HPLC** |
| Remark / Reference : | | | | |

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