**Flow Chart for Analysis of Isoproturon 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. | Mix Cyclo Hexane and Isopropyl alcohol in the proportion of 90:10 (v/v) |  |  |  |
| 3.1.2. | Pass through the 0.45 µm membrane filter under vaccum. |  |  |  |
| 3.1.3. | Homogenize the mixture using the magnetic stirrer. |  |  |  |
| 3.1.4 | Allow to attain room temperature. |  |  |  |
| **3.2.** | **Preparation of Internal Standard Solution:**  |  |  |  |
| 3.2.1 | Weigh 0.25 g of acetanilide in 100 mL volumetric flask. |  |  |  |
| 3.2.2 | *Note down the S.No. of balance log book.* |  |  |  |
| 3.2.3 | Dissolve and make up to the mark with mobile phase(3.1.4) |  |  |  |
| **3.3** | **Preparation of standard solution** |  |  |  |
| 3.3.1 | Note the purity of the standard | % | % |  |
| 3.3.2 | Weigh 0.5 g a. i. of Standard in a 100 ml volumetric flask  | g | g |  |
|  3.3.3 | *Note the serial No. of the balance log book* |  |  |  |
| 3.3.4 | Dissolve in 25 mL of Isopropyl alcohol and make up to the mark with cyclohexane.(Stock A) |  |  |  |
| 3.3.5 | Pipette out 5 mL of Stock A (3.3.4) in to a 50 mL volumetric flask. |  |  |  |
| 3.3.6 | Add 5 mL of internal standard solution (3.2.3) and mix well. |  |  |  |
| 3.3.7 | Make up to the mark with mobile phase (3.1.4). |  |  |  |
| **3.4** |  **Preparation of sample solution** |  |  |  |
| 3.4.1 | Note down the percent active ingredient declared on the sample | % | % |  |
| 3.4.2 | Weigh 0.5 g a. i. of Sample in a 100 ml volumetric flask  | g | g |  |
| 3.4.3 | *Note the serial No. of the balance log book* |  |  |  |
| 3.4.4 | Dissolve in 25 mL of Isopropyl alcohol and make up to the mark with cyclohexane.(Stock B) | ml | ml |  |
| 3.4.5 | Pipette out 5 mL of Stock B (3.4.4) in to a 50 mL volumetric flask. |  |  |  |
| 3.4.6 | Add 5 mL of internal standard solution (3.2.3) and mix well. |  |  |  |
| 3.4.7 | Make up to the mark with mobile phase (3.1.4). |  |  |  |
| 3.4.8 | Filter the sample through 0.45µm filter paper and inject. |  |  |  |
| 4. | **HPLC Parameters** |  |  |  |
| **4.1** | **Column** |  |  |  |
| 4.1.1 | Silica, Particle Size: 5µ  |  |  |  |
| 4.1.2 | Length: 250 mm |  |  |  |
| 4.1.3 | I.D.: 4.6 mm |  |  |  |
| **4.2** | **Mobile Phase** |  |  |  |
| 4.2.1 | Cyclo Hexane : Isopropyl alcohol (90: 10, v/v) |  |  |  |
| 4.2.2 | Flow Rate : 1.5 ml/min |  |  |  |
| **4.3** |  **Detector:** UV |  |  |  |
| **4.4** |  **Wave Length**: 254 nm |  |  |  |
| **4.5** |  **Injection Volume:** 20µl |  |  |  |
| 5. | **Result** |  |  |  |
| Sample chromatogram no.  |  |  |  |
| Standard chromatogram no.  |  |  |  |

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| **6. Calculation:**

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|  Isoproturon content, A1 X A4 X M1 % by mass = ------------------------- x P  A3 X A 2 XM2 | **Where,**M1 =Mass in ‘g’ of Isoproturon standard M2 =Mass in ‘g’ of sample taken for test  A1 = Peak area of Isoproturon in the sample solutionA2 = Peak area of internal standard in the sample solution A3 = Peak area of Isoproturon in the standard solution A4 = Peak area of Internal standard in the standard solution. P = Percent purity of Isoproturon standard |

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**Result:**

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| **Sr. No.** | **Name of test** | **Result** | **Unit** | **Method of Analysis**  |
| 1. | Active ingredient |  | % | IS- 12004-1987(Reaffirmed 2007) |
| Remark / Reference : |

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