**PESTICIDE FORMULATION & RESIDUE ANALYTICAL CENTRE, PMD, NIPHM, HYDERABAD**

Sr. No. in Scope NABL / NON NABL

# Flow chart for analysis of Imidacloprid content in formulation sample

**Date of Analysis**

| Sl. No. | Step | Execution | Executed By |
|---|---|---|---|
| 1. | Sample No. | | |
| 2. | Name of Sample | | |
| 3. | **Procedure** | | |
| **3.1** | **Preparation of Mobile Phase** | | |
| 3.1.1 | Mix acetonitrile and water in the proportion of 80:20 (v/v) | | |
| 3.1.2 | Pass through membrane filter under vacuum | | |
| 3.1.3 | Homogenize the mixture using a magnetic stirrer | | |
| 3.1.4 | Allow to attain room temperature | | |
| **3.2** | **Preparation of Internal Standard Solution** | | |
| 3.2.1 | Weigh 0.03g of Acenaphthene in 100 ml volumetric flask. | g | |
| 3.2.2 | *Note the serial No. of the balance log book* | | |
| 3.2.3 | Add to it 40 ml of mobile phase (3.1.4) | | |
| 3.2.4 | Keep it for 30 minute with intermittent shaking | | |
| 3.2.5 | Dilute 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 0.1g A.I. of standard in 100 ml volumetric flask | g | |
| 3.3.3 | *Note the serial No. of the balance log book* | | |
| 3.3.4 | Add to it 40 ml of mobile phase (3.1.4) | | |
| 3.3.5 | Keep it for 30 minute with intermittent shaking | | |
| 3.3.6 | Dilute up to the mark with mobile phase (3.1.4) | | |
| 3.3.7 | Pipette out 10ml of solution (3.3.6) to a 100 ml volumetric flask | ml | |
| 3.3.8 | Dilute up to the mark with mobile phase (3.1.4) | | |
| 3.3.9 | Pipette out 20ml of solution (3.3.8) to a 100 ml volumetric flask | ml | |
| 3.3.10 | Add 20ml of internal standard solution (3.2.5) | ml | |
| 3.3.11 | Dilute up to the mark with mobile phase (3.1.4) | | |

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| | | | |
|---|---|---|---|
| **3.4** | **Preparation of Sample** | | |
| 3.4.1 | Note the percent active ingredient content declared on sample | % | |
| 3.4.2 | Weigh 0.1g A.I. of sample in 100 ml volumetric flask | g | |
| 3.4.3 | *Note the serial No. of the balance log book* | | |
| 3.4.4 | Add to it 40 ml of mobile phase (3.1.4) | | |
| 3.4.5 | Keep it for 30 minute with intermittent shaking | | |
| 3.4.6 | Dilute up to the mark with mobile phase (3.1.4) | | |
| 3.4.7 | Pipette out 10ml of solution (3.4.6) to a 100 ml volumetric flask | ml | |
| 3.4.8 | Dilute up to the mark with mobile phase (3.1.4) | | |
| 3.4.9 | Pipette out 20ml of solution (3.4.8) to a 100 ml volumetric flask | ml | |
| 3.4.10 | Add 20ml of internal standard solution (3.2.5) | ml | |
| 3.4.11 | Dilute up to the mark with mobile phase (3.1.4) | | |
| **4.** | **HPLC Parameters** | | |
| **4.1** | **Column** | | |
| 4.1.1 | Stainless Steel Packed with Novapak $C_{18}$ | | |
| 4.1.2 | Length: 250 mm | | |
| 4.1.3 | I.D.: 4.6 mm | | |
| **4.2** | **Mobile Phase** | | |
| 4.2.1 | Acetonitrile : Water (80 : 20) | | |
| 4.2.2 | Flow Rate: 1.0 ml/min | | |
| **4.3** | **Detector:** UV | | |
| **4.4** | **Wave Length**: 278 nm | | |
| **4.5** | **Injection Volume:** 20 µl | | |
| **5.** | **Result** | | |
| | Sample chromatogram no. | | |
| | Standard chromatogram no. | | |

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**6. Calculation:**

$$\text{Imidacloprid content, \% by mass} = \frac{A_2 \times A_3 \times M_1}{A_1 \times A_4 \times M_2} \times P$$

**Where,**

$M_1$ = Mass in 'g' of imidacloprid standard
$M_2$ = Mass in 'g' of sample taken for test
$A_1$ = Peak area of imidacloprid in the standard solution
$A_2$ = Peak area of imidacloprid in the sample solution
$A_3$ = Peak area of internal standard in the standard solution
$A_4$ = Peak area of internal standard in the sample solution
P = Percent purity of imidacloprid in the standard

**Result:**

| Sl. No. | Name of test | Result | Unit | Method of Analysis |
|---|---|---|---|---|
| 1. | Active ingredient | | % | 1S 15443 : 2004 (Reaffirmed 2009) |
| Remark / Reference : | | | | |

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

| Name of the Laboratory : | **Pesticide Formulation & Residue Analytical Centre, PMD, NIPHM, Hyderabad** | | | | |
|---|---|---|---|---|---|
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