Doc No. FAD 01(28325)WC
July 2025

BUREAU OF INDIAN STANDARDS

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भारतीय मानक मसौदा

अजाडिरेक्टिन युक्त नीम आधारित पायसनीय सान्द्र — विशिष्टि

(*आइ एस 14300 का पहला पुनरीक्षण*)

Draft Indian Standard

NEEM BASED EC CONTAINING AZADIRACHTIN — SPECIFICATION

(*first revision of IS 14300*)

ICS 65.100.10

Pesticides Sectional Committee, FAD 01	**Last date of comments:** 9 August 2025

FOREWORD

(*Formal clauses would be added later*)

NEEM based EC containing azadirachtin is used for the control of pests of agricultural crops.

This standard was published in 1995. In this revision, the tolerance limits of the declared azadirachtin content have been included and the standard has been brought out in the latest style and format of the Indian Standards, and references to Indian Standards wherever applicable have been updated. It also incorporates three amendments issued to the previous version of this standard.

In the preparation of this standard, due consideration has been given to the provisions of the *Insecticides Act*, 1968 and the Rules framed thereunder. However, this standard is subject to the restrictions imposed under these, wherever applicable.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 2022 'Rules for rounding off numerical values (*second revision*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

1 SCOPE

This standard prescribes the requirements and methods of sampling and test for NEEM based EC containing azadirachtin.

2 REFERENCES

The following standards contain provisions which through reference in this text, constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below:

IS No.	Title
IS 1448 (Part 20) : 2024/ ISO 13736 : 2021	Petroleum and its products — Methods of test: Part 20 Determination of flash point — Abel closed-cup method (*fourth revision*)
IS 2771 (Part 1) : 2022	Corrugated fibreboard boxes — Specification Part 1 General requirements (*third revision*)
IS 6940 : 2025	Pesticides and their formulations — Methods of test (*second revision*)
IS 8190 (Part 2) : 1988	Requirements for packing of pesticides: Part 2 Solid pesticides (*second revision*)
IS 10627 : 1983	Methods for sampling of pesticidal formulations
IS 14299 : 202X	*NEEM* extract concentrate containing azadirachtin — Specification (*first revision*) [*Under preparation Doc No. FAD 01(28090)WC*]

3 REQUIREMENTS

3.1 Constituents

3.1.1 The material shall consist of *NEEM* based azadirachtin, it may be dissolved in suitable solvent(s) together with emulsifying agent(s) and with stabilizer(s).

3.2 Physical

The material shall comply with the following physical requirements.

3.2.1 *Description*

The material shall be homogeneous, stable, light to dark brown viscus liquid with a repulsive odour. It shall readily form an emulsion on dilution with water, suitable for use as spray.

3.2.2 *Cold Test*

When tested by the method prescribed in IS 6940, no turbidity or separation of solid or oily matter shall occur.

3.2.3 *Flash Point*

When tested by the method prescribed in IS 1448 (Part 20), the flash point of the material shall be above 24.5 °C.

3.2.4 *Emulsion Stability*

Any separation including creaming at the top and sedimentation at the bottom of 100 ml of emulsion prepared in standard hard water with 2 ml of concentrate shall not exceed 2 ml when tested by the method prescribed in IS 6940.

3.3 Chemical

The material shall also comply with the following chemical requirements.

3.3.1 *Active Content*

When determined by the method prescribed in Annex A, the observed azadirachtin content, percent (*m*/*m*) of the samples shall not differ from the declared nominal value by more than the tolerance limit indicated below:

Nominal Value, (Percent)	*Tolerance Limit, (Percent)*
Up to 9	+10 -5
Above 9 and below 50	±5 of the nominal value
50 and above	+5 -3

3.3.2 *Acidity* or *Alkalinity*

When tested by the method prescribed in IS 6940, the acidity (as H_2SO_4) or alkalinity (as NaOH) of the material shall not be more than 0.5 percent by mass, respectively.

3.3.3 *Aflatoxin Content*

When tested by the method prescribed in Annex B of IS 14299, no aflatoxin shall he present.

4 PACKING

The material shall be packed in aluminium or HDPE containers. For bulk packing HDPE drums shall be used. The containers shall also comply with the requirements specified in IS 8190 (Part 2).

5 MARKING

5.1 The container shall bear legibly and indelibly the following information:

a) Name of the material;
b) Name and address of the manufacturer;
c) Batch number;
d) Date of manufacture;
e) Date of expiry;
f) Net quantity;
g) Nominal azadirachtin content, percent (*m*/*m*);
h) Cautionary notice as worded in the *Insecticides Act*, 1968, and Rules framed thereunder; and
j) Any other information required under the *Legal Metrology* (*Packaged Commodities*) *Rules*, 2011.

5.2 BIS Certification Marking

The product(s) conforming to the requirements of this standard may be certified as per the conformity assessment schemes under the provisions of the *Bureau of Indian Standards Act*, 2016 and the Rules and Regulations framed thereunder, and the products may be marked with the Standard Mark.

6 SAMPLING

Representative samples of the material shall be drawn as prescribed in IS 10627.

7 TESTS

7.1 Tests shall be carried out by the appropriate methods referred to in **3.2.2** to **3.2.4** and **3.3.1** to **3.3.3.**

7.2 Quality of Reagents

Unless specified otherwise, pure chemicals and distilled water *(see* IS 1070) shall be employed in tests.

NOTE – 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the results of analysis.

ANNEX A

DETERMINATION OF AZADIRACHTIN CONTENT IN *NEEM* BASED FORMULATION EC

A-1 PRINCIPLE

Azadirachtin in the sample is dissolved in methanol-water (90:10) and analyzed on HPLC at 215 nm.

A-2 APPARATUS

A-2.1 High Performance Liquid Chromatography

HPLC unit equipped with ultraviolet (UV) detector and printer-plotter-cum-integrator and operated under the following suggestive parameters. These parameters may be varied as per available facilities provided standardization is done:

Column	C-18, 25 cm x 4.6 mm Stainless Steel 5 µ Particle size or equivalent
Flow rate	1.0 ml/min approx
Detector	Ultraviolet (UV) 215 nm
Mobile phase	Acetonitrile:Water (35:65) (*v*/*v*)
Retention time Azadirachtin – A Azadirachtin – B	 10-12 min (approx) 11-13 min (appox)

The above retention time is required to be confirmed by the respective standard.

A-2.2 Micro Syringe – 25 μl capacity.

A-3 REAGENTS

A-3.1 Reference Standard – Working standard of known purity.

A-3.2 Acetonitrile – HPLC Grade.

A-3.3 Water – HPLC Grade.

A-3.4 Methanol – HPLC Grade.

A-3.5 Volumetric Flasks – 10 ml, 50 ml and 100 ml.

A-3.6 Pipettes – Graduated, 2 ml and 5 ml.

A-4 PREPARATION OF STANDARD SOLUTIONS

A-4.1 Preparation of Reference Standard Solution

Weigh accurately the 2.0 mg working standard of known purity into 50 ml volumetric flask and dissolve in methanol:water (90:10). Make up to the mark and shake well. Take 2 ml of this solution with the help of pipette into a syringe prepared as per **A-4.3**. Elute it into 10 ml volumetric flask with repeated washings with methanol: water (90:10). Make up to the mark, shake well.

A-4.2 Preparation of Sample Solution

Weigh accurately sample quantity so as to contain 2.0 mg of azadirachtin in 50 ml volumetric flask and dissolve in methanol: water (90:10). Shake well and keep it aside to separate the layers. Take 2 ml of this solution with the help of pipette into a syringe prepared as per **A-4.3**. Elute it into 10 ml volumetric flask with repeated washings with methanol and water (90:10). Make up to the mark and shake well.

A-4.3 Preparation of Thin Column of CS Material in Syringe

Take a sep pak cartridge containing 0.5 g of C18 packing material and wet it with solvent methanol:water (90:10) before loading the sample solution. Alternatively, take a 5 ml glass syringe of 10 mm internal diameter and insert a small wad of silanised glass wool inside the syringe to the bottom. Add C18 powder (30-40 μm) to form about 1 cm height column (approx 0.5 g), put another wad of glass wool and wet with solvent (methanol : water 90:10).

A-5 ESTIMATION

A-5.1 Inject 20 μl of working standard and sample solution respectively to get area reproducibility for two consecutive injections. The area of two consecutive injections should not vary by more than 2 percent. From the HPLC chromatogram calculate percentage of azadirachtin in the sample as in **A-5.2**.

A-5.2 Calculation

$$\text{Azadirachtin content, content, percent by mass} = \frac{A_1 \times M_2}{A_2 \times M_1} \times P$$

where,

A_1 = peak area of azadirachtin in sample solution (*Aza A + B*);
A_2= area of azadirachtin in working standard (*Aza A + B*);
M_1 = mass, in g, of the sample taken for test;
M_2 = mass, in g, of the working standard azadirachtin; and
P = purity of working standard.

A-5.3 Analytical Tolerance

Adopt analytical tolerance after analyzing the fortified samples of oil formulations, if necessary (normally the tolerance level is 10-15 percent).