भारतीय मानक

कीटनाशी — कृषि, खाद्य वस्तुओं, मिट्टी एवं पानी में मेटालेक्सिल ज्ञात करने की पद्धति

Indian Standard

PESTICIDE — DETERMINATION OF METALAXYL RESIDUES IN AGRICULTURAL, FOOD COMMODITIES, SOIL AND WATER

UDC 664 : 543·544·25 [632·952·028 MET]

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BUREAU OF INDIAN STANDARDS
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July 1994

Price Group 2

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards after the draft finalized by the Pesticides Residue Analysis Sectional Committee had been approved by the Food and Agriculture Division Council.

Metalaxyl formulations are used as fungicides in agriculture. This standard will enable the food, health authorities and others engaged in the field to follow uniform test procedure for the estimation of residues of metalaxyl in various agricultural and food commodities.

In the preparation of this standard, due consideration has been given to the provisions of *Prevention of Food Adulteration Act, 1954* and Rules framed thereunder and *Standards of Weights and Measures (Packaged Commodities) Rules, 1977.*

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values *(revised)*'.

Indian Standard

PESTICIDE — DETERMINATION OF METALAXYL RESIDUES IN AGRICULTURAL, FOOD COMMODITIES, SOIL AND WATER

1 SCOPE

1.1 This standard prescribes a gas chromatographic method for determination of residues of metalaxyl [methyl N - (2 - methody - acetyl)-N-(2,6-xylyl)-DL-alaninate] in agricultural and food commodities.

1.2 This method has a detection limit lower than of 0.05 μg/g (0.05 ppm) using a alkali-flame ionization detector.

2 REFERENCES

The Indian Standards listed at Annex A are necessary adjuncts to this standard.

3 QUALITY OF REAGENTS

Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070 : 1992) shall be employed in tests.

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

4 SAMPLING

The representative samples for the purpose of estimating metalaxyl residues in the samples shall be drawn in accordance with IS 11380 : 1985.

5 PRINCIPLE

Soil samples are extracted with methanol in a hot extractor. Plant material is extracted with methanol in a high speed homogenizer. The methanol extracts are diluted with water and cleaned up by water-methanol/dichloromethane partitioning. The dichloromethane phases are evaporated and the residue of the organic phases are cleaned up by alumina column chromatography before the final determination by gas chromatography.

6 SAMPLE STORAGE

Sample shall be stored as such when chances of its degradation do not exist and the quantity is small. Otherwise extraction shall be carried out and the extract stored. Depending upon the nature of the sample, keep the sample or its extract either in deep-freezer at −15°C or in refrigerator until taken up for analysis. Ensure that the samples do not absorb or loose moisture during storage. Avoid undue long storage period.

7 APPARATUS

7.1 Gas Chromatograph

The gas chromatograph shall be fitted with an alkali-flame inoization detector (AFID) and printer — plotter-*cum*-integrator. The following operating parameters are suggested, which can be changed provided standardization is done :

Alkali-Flame Inoization Detector (AFID)

Column: Glass, 100 cm length × 2 mm I.D.; packed with 3% carbowax 20 M on Gas Chrom Q DMCS treated (size: 0.15 - 0.18 mm)

Temperature:

Column Oven	185°C
Injection Port	240°C
Detector	240°C

Carrier Gas and Flow Rate : Nitrogen; 35 ml/min

Fuel Gas and Flow Rate : Hydrogen; 35 ml/min

Air and Flow Rate: 230 ml/min

7.1.1 *Microlitre Syringe* — 10 μl capacity.

7.1.2 *Warning Blender or Equivalent*

7.1.3 *Centrifuge*

7.1.4 *Rotary Evaporative Concentrator*

7.1.5 *Sample Grinder*

7.1.6 *Mechanical Shaker*

7.1.7 *Ultrasonic Bath*

7.1.8 *Chromatographic Column* — glass, 200 cm × 18 mm I.D. with ground glass top.

7.2 Reagents

7.2.1 *Methanol* — glass re-distilled.

7.2.2 *Saturated Sodium Chloride Solution* — AR grade.

7.2.3 n-*hexane* — glass re-distilled.

7.2.4 *Diethyl Ether* — glass re-distilled.

7.2.5 *Dichloromethane* — glass re-distilled.

7.2.6 *Reference Standard Metalaxyl* — of known purity.

7.2.7 *Sodium Hydrogen Carbonate* — AR grade.

7.2.8 *Alumina Acidic* — W 200, activity grade V (19% water added).

8 EXTRACTION

8.1 Vegetables

Shred the entire sample with food cutter. Weigh 100 g representative sample into a 500 ml wide-mouth jar and add 200 ml methanol. Macerate with the high speed blender for 2-3 minutes. Shake the bottle for 2 hours on a mechanical shaker. Filter through Whatman 40 or equivalent filter paper on a Buchner funnel, under suction. Rinse the jar and wash the filter-cake twice with 40 ml of methanol each time. Adjust the volume to 400 ml and take an aliquot of 200 ml corresponding to 50 g sample.

8.2 Grapes

Weigh 100 g representative sample into a 500 ml wide mouth jar and add about 2 g of sodium hydrogen carbonate, 200 ml methanol and macerate with the high speed blender for 2-3 minutes. Check the *p*H value to be above 7 otherwise add more sodium hydrogen carbonate. Shake the bottle for 2 hours on a mechanical shaker. Filter through Whatman 40 or equivalent filter paper on a Buchner funnel, under suction. Rinse the jar and wash the filter-cake twice with 40 ml of methanol each time. Adjust the volume to 400 ml and take an aliquot of 200 ml corresponding to 50 g sample.

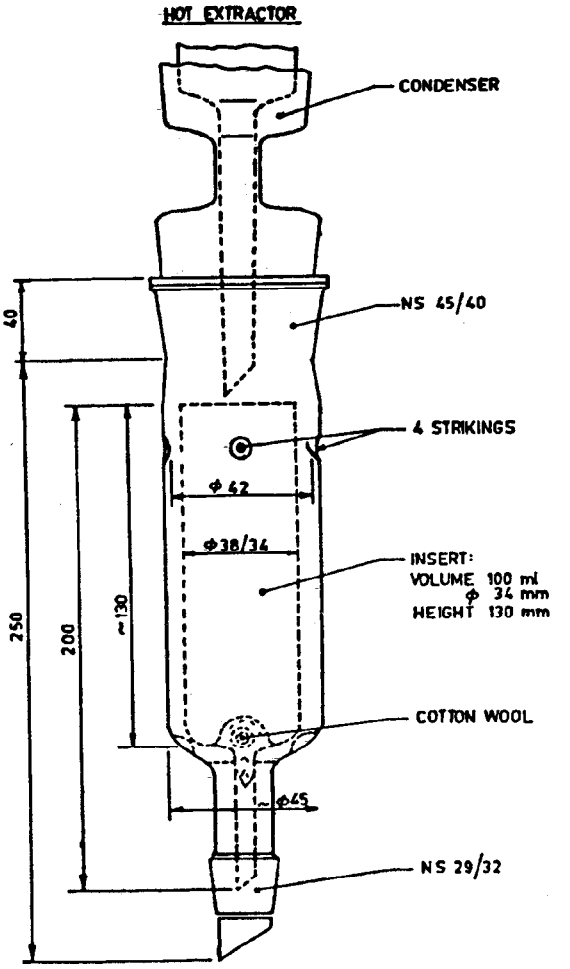
8.3 Soil

Remove big stones from the sample. Homogenize the sample in a planetory blender. Weigh 50 g representative sample into the glass insert of the hot extractor (Fig. 1). Moisten the dry sample with 10 ml of distilled water. Extract with 150 ml methanol for 2 hours.

NOTE — Samples with high stone content cannot be homogenized in the planetary blender. Air dry those samples then grind in cross-beater mill and mix thoroughly.

9 PARTITIONING

Transfer the corresponding extract-aliquots to a 100 ml separatory funnel and add 200 ml water and 20 ml saturated sodium chloride solution. Extract the aqueous solution 3 times with 75 ml of dichloromethane by vigorously shaking the separatory funnel during each extraction. Collect the dichloromethane phases and filter through a plug of cotton and evaporate to dryness using a rotating evaporator at 40°C.



All dimensions in millimetres.

Fig. 1 METALAXYL EXTRACTION APPARATUS

10 CLEAN-UP

10.1 Fill the chromatographic column with *n*-hexane. Add alumina acidic to a height of 7 cm (30 ml of volume). Drain the solvent to the top of the alumina. Dissolve the residue from partitioning in 5 ml of *n*-hexane by immersing the flask into the ultrasonic bath for 3 minutes. Transfer this solution to the column. Rinse the flask twice with 5 ml of *n*-hexane and transfer each portion to the column. Rinse the flask and then the column with 100 ml of *n*-hexane. Rinse the flask and then the column with 30 ml of a mixture of *n*-hexane and diethyl ether (1:1). Discard both eluates. Elute the active ingredient with further 80 ml of the mixture of *n*-hexane and diethyl ether (1:1). Collect the eluate and evaporate to dryness in a rotation evoporator at 40°C.

10.2 Transfer the residue with 3 increments each of 5 ml diethyl ether to 25 ml vials and evaporate to dryness in a stream of clean air. Dissolve the residue for gas chromatographic determination in 1 ml *n*-hexane-ethanol (1:1) mixture.

11 ESTIMATION

11.1 Preparation of Standard Curve

Prepare solutions containing 2.5 to 10 μg reference standard metalaxyl in one ml of *n*-hexane-ethanol (1:1) mixture. Inject a minimum of 4 different amounts of metalaxyl ranging from 2.5 to 10 mg for AFID detector. Measure the peak areas and plot them against the active ingredient on a log-log scale.

11.2 Inject into the gas chromatograph, with the help of a microlitre syringe, a suitable aliquot of the sample, identify the peaks by the retention times and measure the peak areas. Estimate the metalaxyl content by comparison with the standard curve.

12 CALCULATION

$$\text{Metalaxyl residue, } \mu g/g = \frac{A_1 \times V_2 \times V_3 \times C}{A_2 \times V_1 \times M} \times f$$

where

A_1 = peak area of the sample;

V_2 = volume, in μl of standard metalaxyl solution injected;

V_3 = total volume, in ml, of sample solution;

C = concentration, in μg/g, of the standard metalaxyl solution;

f = recovery factor $= \dfrac{100}{\text{percent mean recovery}}$

A_2 = peak area of standard metalaxyl;

V_1 = volume, in μl, of sample solution injected; and

M = mass, in g, of sample taken for analysis.

NOTE — Percent mean recovery is determined by taking untreated control sample to which known amounts of metalaxyl standard is added and analysed as described above.

ANNEX A

(*Clause* 2)

LIST OF REFERRED INDIAN STANDARDS

IS No.	*Title*	*IS No.*	*Title*
1070 : 1992	Reagent grade water *(third revision)*	11380 : 1985	Methods of sampling for determination of pesticide residues in agricultural and food commodities

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This Indian Standard has been developed from Doc: No. FAD 34 (83)

Amendments Issued Since Publication

Amend No.	Date of Issue	Text Affected

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