भारतीय मानक

कृषि एवं खाद्य पदार्थ, भूमि एवं जल में फोरेट अवशेष ज्ञात करने की विधि

Indian Standard

METHOD FOR DETERMINATION OF PHORATE RESIDUES IN AGRICULTURE AND FOOD COMMODITIES, SOIL AND WATER

ICS 13.080.99; 65.100.01; 67.040; 71.040.50

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BUREAU OF INDIAN STANDARDS
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NEW DELHI 110002

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Price Group 2

Pesticide Residues Analysis Sectional Committee, FAD 34

FOREWORD

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

Phorate, 0,0-dimethyl S-ethylthiomethyl phosphorodithioate, formulations are extensively used in agriculture for the control of many insect pests. 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 phorate in agriculture and food commodities, soil and water.

In preparation of this Standard due consideration has been given to the limits of phorate residues which have been laid down under the provisions of *Prevention of Food Adulteration Act,* 1954 and Rules framed thereunder and *Standards of Weights and Measures (Packaged Commodities) Rules*, 1977. The specified test method is sensitive to the prescribed level of residues.

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

METHOD FOR DETERMINATION OF PHORATE RESIDUES IN AGRICULTURE AND FOOD COMMODITIES, SOIL AND WATER

1 SCOPE

This standard prescribes gas chromatographic method for determination of residues of phorate (0,0-dimethyl S-ethylthiomethyl phosphorodithioate) and its toxic metabolites phorate oxon sulfoxide in agriculture and food commodities, soil and water. The method is applicable with a limit of determination in the range of 0.01 µg/g (ppm).

2 REFERENCES

The following Indian 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*
1070 : 1992	Reagent grade water (*third revision*)
11380 (Part 1) : 1985	Method of sampling for determination of pesticide residues : Part 1 In agricultural and food commodities

3 PRINCIPLE

Residues of phorate and its all toxic metabolites are extracted with acetone. The acetone extract is concentrated, diluted with water and residues are partitioned into ethyl acetate. The ethyl acetate extract is concentrated and cleaned up by column chromatography using 2 : 5 (*w*/*w*) mixture of Darco G 60 and cellulose powder. After clean up, the extract is oxidised to convert all active residues into sulfones, which is then measured by gas chromatography with thermionic or alkali flame ionization detector.

4 QUALITY OF REAGENTS

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

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

5 SAMPLING

The representative samples for the purpose of estimating phorate residues in agriculture and food commodities, soil and water shall be drawn in accordance with IS 11380 (Part 1).

6 APPARATUS

6.1 Mechanical Shaker

6.2 Vacuum Rotary Evaporator

6.3 Chromatographic Column

Glass 40 cm × 2 cm.

6.4 Buchner Funnel

6.5 Erlenmeyer (Conical) Flask

500 ml, 250 ml capacity.

6.6 Round Bottom Boiling Flasks

500 ml, 250 ml, 100 ml capacity.

6.7 Separatory Funnel

1 000 ml, 500 ml, 125 ml capacity.

6.8 Volumetric Flask

200 ml, 100 ml capacity.

6.9 Gas Chromatograph

A suitable gas chromatograph equipped with a thermoionic or alkali flame ionization detector or NPD and FPD and operating under the following suggested parameters. These parameters may be varied according to the available facilities, provided standardization is done:

Column :	Glass 3.5 ft × ¼ inch packed with 3.5% DC 200 and 1.5% QF-1 on 80-100 mesh Gas Chrom Q	
Temperatures :	Column oven	200°C
	Injection port	225°C
	Detector	240°C
Gas Flow Rates :	Nitrogen (carrier gas)	30 ml/min
	Hydrogen	3-5 ml/min
	Air	300 ml/min
Retention Time :	Phorate sulfone	5.9 min (approx)
	Phorate oxon sulfone (oxygen along)	4.7

6.10 Microlitre Syringe

10 µl capacity.

7 REAGENTS

7.1 Acetone

Glass redistilled.

7.2 Ethyl Acetate

Glass redistilled.

7.3 Chloroform

Glass redistilled.

7.4 Methanol

Glass redistilled.

7.5 Sodium Sulphate

Anhydrous.

7.6 Sodium Chloride

AR Grade.

7.7 Darco G 60

Active charcoal, acid washed.

7.8 Cellulose Powder for Column Chromatography

60-120 mesh.

7.9 Megnesium Sulphate

AR Grade.

7.10 Sodium Sulphate

Anhydrous.

7.11 Sodium Chloride

AR Grade.

7.12 Potassium Permanganate

AR Grade.

7.13 Phorate Reference Standard

Of known purity.

7.14 Phorate Oxon Reference Standard

Of known purity.

7.15 Preparation of Standard Solution

Weigh 0.1 g of phorate and 0.1 g of phorate oxon (oxygen analog) in a 100 ml volumetric flask. Make to volume with acetone. Transfer 1 ml of this solution to a 200 ml volumetric flask, make to volume with acetone and mix. This flask contains 5 µg/ml each of phorate and phortate, and phorate oxygen analogue.

8 EXTRACTION

8.1 Plant Material and Soil

Take 25 g of finely chopped plant, fruit or vegetable, finely ground grain sample or soil sample in a 250 ml conical flask. Add 100 ml of acetone and extract by shaking the contents on a mechnaical shaker at slow to moderate speed for 2 h. Filter the contents through suction into 500 ml suction flask and repeat the extraction twice with 75 ml each portions of acetone collecting the acetone extract each time. Combine the acetone extrtacts, transfer into a 500 ml round bottom flask and concentrate to about 50 ml in vacuum rotary evaporator.

Transfer the concentrated extract to 1 litre saparatory funnel dilute with 50 ml saturated sodium chloride solution followed by 300 ml distilled water. Extract this aqueous phase with three 100-ml portions of the ethyl acetate. Collect the ethyl acetate extract through a layer of anhydrous sodium sulphate kept over a funnel with a glass wool pad. Combine the ethyl acetate extract, transfer to a 500 ml round bottom flask and concentrate to dryness using a rotary vacuum evaporator.

8.2 Water Samples

Place an appropriate volume of the water sample (9 150 to 200 ml) in a 500 ml separatory funnel and extract thrice with 100 ml portions of ethyl acetate, passing the extract through a layer of anhydrous sodium sulphate. Transfer the dried extract to a 500 ml round bottom flask and concentrate to dryness using a rotary vacuum evaporator.

9 CLEAN UP

Prepare a chromatographic column in the following order in distinct layers — a layer of glass wool, 1.5 cm of anhydrous sodium sulphate; 4 cm of 2:5 (*w/w*) mixture of Darco G 60 — celllulose powder, 1.5 cm of anhydrous sodium sulphate using ehtyl acetate as the solvent. Wash the column with 20 ml of ethyl acetate followed by 20 ml of hexane. Do not allow the solvent to fall to level below the upper layer of adsorbent packing.

Dissolve the concentrated extract obtained as in **8.1** or **8.2** in 2 ml of ethyl acetate and transfer quantitatively into the column using three 2-ml washings with ethyl acetate. Elute the column with 100 ml of 20 percent methanol in ethyl acetate, collect the eluate in 250 ml round bottom flask and concentrate to dryness using a rotatry vacuum evaporator.

10 OXIDATION

Place 2 ml of the standard (*see* **7.13**) solution in a 100 ml round bottom flask. This standard is carried

through the reminder of the procedure and contains 5 µg/ml each of phorate, and phorate and phorate oxygen analogue.

Dissolve the sample residues from the previous step in 2 ml of acetone. Add 5 ml of 20 percent (*w/v*) magnesium sulphate solution and 25 ml of 0.5 N $KMnO_4$ solution, washing down the sides of the flask during the addition. Mix and let stand for 30 min with occasional swirling for 30 min, making sure that there is an excess of permanganate the entire time. Transfer the oxidation chloroform and add this to separatory funnel containing the oxidation mixture. Shake the separatory funnel for 30 s to extract, allow the phases to separate (centrifuge if necessary), and drain the lower phase through 15 to 20 g of powdered, anhydrous sodium sulphate retained in a powder funnel with a loose plug of glass wool. Collect the filtrate in a 250-ml round bottom flask. Repeat the above extraction twice more with fresh 25 ml portions of chloroform. After the final extraction, rinse the sodium sulphate with 20 ml of chloroform. Evaporate the combined extracts just to dryness on a rotary vacuum evaporator at 40°C. Remove any last traces of solvent with a stream of dry air at room temperature. Dissolve the residue in ethyl acetate and analyze by GLC as the sulfones.

11 ESTIMATION

Dissolve the standard and sample residue from the previous oxidation step (*see* **10**) suitable in ethyl acetate and inject an appropriate aliquot of the standard or sample solution into the gas chromatograph. Identify the phorate sulfone and the oxygen analogue sulfone peaks by their retention times and measure the peak areas.

12 CALCULATION

Calculate the parts per million of residue 9 µg/g in sample by comparing the response (peak area) obtained for an unknown to the response obtained for a known amount of phorate or oxygen analogue standard carried through the procedure from the oxidation step. The standard is a composite of both compounds, but parts per million in the unknowns are calculated separately using the corresponding standard peak, because chromatography response is slightly different for the two compounds.

Residues of phorate/oxygen analogue

$$(\mu g/g) = \frac{A_s}{A_{std}} \times C \times \frac{V_{std}}{V_s} \times \frac{V}{M} \times F$$

where

A_s = peak area of the sample

A_{std} = peak area of the standard

C = concentration, in µg/ml, of the standard solution

V_{std} = volume in µl, of standard injected

V_s = volume in µl, of sample solution injected

V = total volume, in ml, of the sample solution

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

F = recovery factor $= \frac{100}{\text{Percent mean recovery}}$

NOTE — Percent mean recovery is determined by taking untreated control sample to which a known amount of phorate and its oxygen analogue is added and analysed as described above.

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