भारतीय मानक

(Reaffirmed 1998)

कीटनाशी — कृषि और खाद्य पदार्थों, मिट्टी एवं पानी में अवशिष्ट ज्ञात करने की पद्धति — एसेफेट एवं उसके टाक्सिक मेटाबॉलाईट, मेथामिडोफॉस

Indian Standard

PESTICIDE — METHOD FOR DETERMINATION OF RESIDUES IN AGRICULTURAL AND FOOD COMMODITIES, SOIL AND WATER — ACEPHATE AND ITS TOXIC METABOLITE, METHAMIDOFOS

UDC 664 : 543 [632'95'028 ACE]

BUREAU OF INDIAN STANDARDS
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NEW DELHI 110002

August 1993

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 Agri-. culture Division Council.

Acephate (0, S-dimethyl acetyl phosphoramidothioate) is used in agriculture for the control of insect pests. Assessment of its residues in food commodities is an important step in sefeguarding human health.

This standard will enable the health authorities and others engaged in the field to follow uniform test procedures for the estimation of acephate and/or methamidofos residues in food commodities. In the preparation of this standard, due consideration has been given to the maximum limits of' acephate and/or methamidofos residues laid under the provisions of ***Prevention of Food Adulteration Act, 1954*** and the Rules framed thereunder. The test method is restricted to the prescribed level of residues.

In reporting the result 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 -METHOD FOR DETERMINATION OF RESIDUES IN AGRICULTURAL AND FOOD COMMODITIES, SOIL AND WATER — ACEPHATE AND ITS TOXIC METABOLITE, METHAMIDOFOS

1 SCOPE

1.1 This standard prescribes the gas chromatographic (GLC) method for determination of residues of acephate and/or its principal toxic metabolite, methamidofos (O, S-dimethyl phosphoramidothioate) in agriculture and food commodities, soil and water.

1.2 The method is applicable with a limit of detection in the range of 0·01 μg/g (ppm) for acephate and 0'02 μg/g (ppm) for methamidofos.

1.3 Though no set procedure for thin layer chromatography (TLC) is being prescribed, standardized TLC procedures may be followed, if necessary, for the purpose of clean up, identification and confirmation of residues of acephate and/or methamidofos.

2 REFERENCES

The Indian Standards listed below are necessary adjuncts to this standard:

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

3 PRINCIPLE

The residues of acephate and its toxic metabolite, methamidofos extracted from the sample and subjected to the clean up step, are estimated gas chromatographically (GLC) in an instrument equipped with thermoionic or flame photometric detector. The contents of acephate and methamidofos are determined by comparing the instrument response with those of the standards of similar concentrations. In this method, residue levels of both acephate and methamidofos can be estimated separately based on the two GLC peaks.

4 QUALITY OF REAGENTS

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

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

5 SAMPLING

The representative samples for the purpose of estimating residues of acephate in food commodities shall be in accordance with IS 11380 : 1985.

6 APPARATUS

6.1 Waring Blender

6.2 Vacuum Rotary Evaporator

6.3 Cbromatographic Column

Glass 30cm x 2 cm.

6.4 Gas Chromatograph

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

Column	: Glass, 100 cm long x 0'4 cm i.o.d packed with 5 percent OV-101 on chromosorb G		
Temperatures	: Column oven	190°C	
	Injector	225°C	
	Detector	240°C	
Carrier gas (nitrogen) flow rate	: 30 ml/min		
Retention time	: Methamidofos	2'2 min (approx)	
	Acephate	3'1 min (approx)	

6.5 Microlitre Syringe

10 μl capacity.

7 REAGENTS

7.1 Ethyl Acetate

Glass redistilled.

7.2 Ethyl Ether

AR Grade.

7.3 Methanol
Glass redistilled.

7.4 **Acetonitrile**
Glass redistilled.

7.5 **Sodium Sulpbate**
Anhydrous.

7.6 **Silica Gel for Column Chromatography**
60- 120 mesh.

7.7 **Petroleum Ether**
Boiling point 40-60°C.

7.8 **Methanol in Ethyl Ether**
5 percent (*v*/*v*).

7.9 **Methyl Isobutyl Ketone**

7.10 Methanol in Ethyl Ether
10 percent (*v*/*v*).

7.11 **Acephate** Reference Standard
Of known purity.

7.12 Metbamidofos Reference Standard
Of known purity.

7.13 **Aqueous Sodium Chloride Solution**
5 percent (*m*/*v*).

7.14 Metbylene Cbloride
AR grade.

8 EXTRACTION

8.1 Fruit **and Vegetables**

Transfer 50 g of finely chopped fruit or vegetable sample to a waring blender. Add 150 g anhydrous sodium sulphate, mix well add 150 ml ethyl acetate. Blend the mixture for 5 minutes. Filter the extract through a layer of anhydrous sodium sulphate on a filter paper mounted on the funnel. Transfer the residues on the filter paper back to the waring blender, add 100 ml ethyl acetate, blend for 5 minutes and filter again. Rinse the residue and the filter twice with 50 ml portion of ethyl acetate. Collect the filtrates and washings and evaporate the combined extracts to dryness in a vacuum rotary evaporator using water bath at 40-60°C.

8.2 Cereals and Grains

Transfer 50 g finely ground sample to a waring blender, add about 20 g anhydrous sodium sulphate followed by 150 ml ethyl acetate. Blend the mixture for 5 minutes and continue the extraction as described in 8.1.

8.3 Oilseeds and Nuts

Transfer 50 g of finely powdered sample to a waring blender, add about 50 g anhydrous sodium sulphate, mix well and add 150 ml ethyl acetate. Blend the mixture for 5 minutes and continue the extraction as prescribed in 8.1. After evaporation of the combined extract dissolve the residue in 50 ml acetonitrile and transfer the solution to a 125-ml separator-y funnel. Rinse the flask twice with 10 ml portion of acetonitrile adding the washing to the separatory funnel. Wash the acetonitrile solution thrice with 25 ml petroleum ether discarding the washings. Finally evaporate the acetonitrile solution to dryness in a vacuum, rotary evaporatory using water bath at 40-60°C.

8.4 **Soil**

Transfer 50 g of air dried and sieved soil into a 500-ml conical flask. Add about 200 ml of acetone. Shake the contents well on a rotary shaker for two hours. Filter extract through Buchner funnel with 20 ml additional acetone. Collect the extracts and evaporate off solvent on a vacuum rotary evaporator. Add about 100 ml of 5 percent aqueous sodium chloride solution (see 7.13) to a 250-ml separatory funnel. Dissolve the residues in 50 ml of methylene chloride and transfer the solution to the separatory funnel. Rinse the flask twice witb 10 ml portions of methylene chloride, add the washings to the separatory funnel. Shake the contents in separatory funnel well for about 2 minutes. Allow the layers to separate. Drain off the lower methylene chloride layer through a layer of anhydrous sodium sulphate mounted on a funnel. Repeat the partitioning twice with 50 ml portions of methylene chloride. Wash the layer of sodium sulphate with 20 ml additional methylene chloride. Collect the methylene chloride extracts and finally evaporate to dryness in a vacuum rotary evaporator using water bath at 40-60°C.

8.5 **Water**

Transfer 300 ml of the water to a 250-ml separatory funnel. Add 50 ml of aqueous sodium chloride solution (see 7.13) followed by 75 ml of methylene chloride to the separatory funnel. Shake the contents well for about 2 minutes and allow the layer to separate. Drain off the lower organic layer through a layer of anhydrous sodium sulphate mounted on funnel. Repeat the extraction twice with 50 ml portions of methylene chloride. Wash the sodium sulpate layer with 10 ml additional methylene chloride. Collect the methylene chloride extracts and evaporate off to dryness in a vacuum rotary evaporator using water bath at 40-60°C.

9 CLEAN UP

9.1 Prepare a pre-washed silica gel chromatographic column by placing a glass wool in the

bottom of the column, add 50 ml ethyl ether and 15 g silica gel. Rinse the sides of the column with ether and place a 15 g layer of sodium sulphate over the silica gel. Again rinse the sides of the column with ether and allow the relevant to drain to the top of the column.

9.2 Using several 5 ml portions of ether, transfer the extracted residue (see 8.1-8.5) quantitatively or to the column. Wash the column with 100 ml ether followed by 100 ml -of 5 percent methanol in ether (see 7.8) discarding the eluate. Then elute acephate and methamidofos from the column with 200 ml 10 percent methanol in ether (see 7.10). Collect the eluate and evaporate to dryness in vacuum rotary evaporatory.

10 ESTIMATION

10.1 Preparation of Standard Solution

For reference standard, prepare solutions of acephate and methamidofos in methyl isobutyl ketone with concentrations of 0'01 to 10 μg/ml and measure the peak area after each injection.

10.2 Preparation of Sample Solution

Dissolve the residue after clean up (see 9.2) in 2 ml methyl isobutyl ketone and inject 2 μl of this solution into the gas chromatograph. ldentify the peaks for methamidofos and acephate by their retention times and measure the peak area.

11 CALCULATION

Residue of captafol (μg/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 captafol injected;

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

c = concentration, in μg/g, of the standard solutions;

f = recovery factor

$$= \frac{100}{\text{percent mean recovery}},$$

A_2 = peak area of the standard;

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

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

NOTE — Percent mean recovery is determined by taking untreated control sample to which a known amount of acephate methamidofos is added and analyzed as described above.

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