IS 14922 (2001): Multiresidue Methods for the Determination of Organo Phosphorus Compounds (Monochrotophos, Anilophos, Phosalone, Temphos, Isazophos, Triazophos and Chlorpyrifos) [FAD 1: Pesticides and Pesticides Residue Analysis]

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IS 14922 : 2001
(Reaffirmed - 2012)

भारतीय मानक

कार्बोनिक यौगिक के अवशेष ज्ञात करने की बहुद्देशीय विधि (मोनोक्रोटोफास, एनीलोफास, फोसेलोन, टेमफोस, इसाजोफास, ट्राइजोफास और क्लोरपाइरीफास)

Indian Standard

MULTIRESIDUE METHODS FOR THE DETERMINATION OF ORGANOPHOSPHORUS COMPOUNDS (MONOCROTOPHOS, ANILOFOS, PHOSALONE, TEMEPHOS, ISAZOPHOS, TRIAZOPHOS AND CHLORPYRIFOS)

ICS 65.100.01;67.040, 71.040.50

BUREAU OF INDIAN STANDARDS
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June 2001

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 Agricultural Division Council.

In preparation of this standard due consideration has been given to the limits of organophosphorus compounds, such as monocrotophos, anilofos, phosalone, temephos, isazophos, triazophos and chlorpyrifos which have been laid down under the provisions of *Prevention of Food Adulteration Act,* 1954 and Rules framed thereunder. The specified test methods are sensitive to the prescribed levels of residue.

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

MULTIRESIDUE METHODS FOR THE DETERMINATION OF ORGANOPHOSPHORUS COMPOUNDS (MONOCROTOPHOS, ANILOFOS, PHOSALONE, TEMEPHOS, ISAZOPHOS, TRIAZOPHOS AND CHLORPYRIFOS)

1 SCOPE

This standard prescribes a HPLC/GLC method for the determination of organophosphorus pesticide (multiresidue method) residues at sub ppm levels in food commodities, after appropriate sample preparation and clean-up.

2 REFERENCE

The following Indian Standard contains provisions which through reference in this text, constitutes provision of this standard. At the time of publication, the edition indicated was 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 edition of the standard indicated below:

IS No.	*Title*
11380 (Part 1) : 1985	Method of sampling for the determination of pesticide residues : Part 1 Agricultural and food commodities

3 SAMPLTING

3.1 The representative sample for the purpose of estimating organophosphorus pesticide residues (multiresidue method) in food commodities shall be in accordance with the sampling procedures as prescribed in IS 11380 (Part 1).

4 APPARATUS AND REAGENTS

4.1 High Speed Sumeeth Blender or Equivalent

4.2 Buch Rotary Vacuum Evaporator

4.3 Mechanical Shaker

4.4 Gas Chromatograph with Thermal Ionic Detector (TID)

4.5 5 percent SE-30 on Chromosorb WHP (100-120 Mesh) 2 m × 1/8" Stainless Steel Column Size 2 m × 2 nm

4.6 Isocratic HPLC System with UV Detector at 225 nm

4.7 RP-18 (25 cm × 0.46 cm, i.d.) 5 μ Particle Size, Stainless Steel Column

4.8 Round Bottom Flask

4.9 Buchnor Funnel and Flask

4.10 Separatory Funnel — 1 000 ml.

4.11 Graduated Pipettes — 1 ml and 10 ml.

4.12 Graduated Cylinders — 10 ml, 250 ml.

4.13 Glasswool

4.14 Stoppered Bottles — 500 ml.

4.15 Glass Column of 2 cm × 30 cm internal dia

4.16 Acetone — AR grade.

4.17 Acetonitrile — AR grade.

4.18 *n*-Hexane — AR grade.

4.19 Ethyl acetate — AR grade.

4.20 Sodium Sulphate (Anhydrous) — AR grade.

4.21 Methanol — HPLC grade.

4.22 Water — HPLC grade.

4.23 Celite 545

4.24 Florisil — 60-100 mesh.

5 PROCEDURE

5.1 Extraction

5.1.1 *Food Grains*

Macerate 50 g of the grain or cereal may be blended for 3 minutes in a high speed blender and extract it with

100 ml of acetone in a mechanical shaker. Filter the acetone layer. Repeat the extraction with another aliquot of 100 ml acetone. Combine the filtrates from both the extractions and concentrate to approximately 25 ml in a rotary vacuum evaporator for 30 minutes time.

5.1.2 *Vegetables, Fruits and Other High Moisture Products*

Macerate 50 g of the sample in a high speed blender, add 5 g of celite 545 and extract with 100 ml of acetone in a mechanical shaker. Filter the acetone layer and give one more extract of another 100 ml acetone to the sample. Combine the filtrates from both the extractions and concentrate to approximately 25 ml in a rotary vacuum evaporator.

5.1.3 *Soil and Seed Crops*

Macerate 50 g of the sample in a high speed blender and extract with 100 ml of acetonitrile in a mechanical shaker. Filter the acetonitrile extract and repeat the extraction with another aliquot of 100 ml acetonitrile. Combine the filtrates from both extractions and add 50 ml of *n*-hexane to the acetonitrile filtrate. Shake the layers vigorously, and, after 2 minutes of shaking, discard the hexane layer. Repeat the process twice, discarding hexane layer each time. Concentrate the acetonitrile layer to 25 ml with the help of rotary vacuum evaporator.

5.2 Partitioning (common for 5.1.1, 5.1.2 and 5.1.3)

Add 100 ml of 10 percent sodium chloride solution to the acetone/acetonitrile layer obtained at the end of the extraction step. Add to this, 100 ml of ethyl acetate and extract. Collect the organic layer (ethyl acetate) and repeat the extraction step using another 100 ml of ethyl acetate (extraction of equivalent layer). Combine both the organic layers and pass it through anhydrous sodium sulphate (20 g). Concentrate the ethyl acetate layer to small (3-5 ml) using rotary vacuum evaporator.

6 COLUMN CHROMATOGRAPHY (common for 5.1.2 and 5.1.3)

Take 1 cm i.d. glass column fitted with a teflon stopcock and pack the bottom of this column using glass wool, add a slurry of 2 g of anhydrous sodium sulphate in *n*-hexane, followed by a slurry of 7 g of florisil and again 2 g of anhydrous sodium sulphate. Bring the level of the solvent in the column to its bed height. Transfer the sample solution obtained at the end of partitioning step into the column and elute the column with 120 ml of ethyl acetate: hexane (30:70) mixture. Collect the eluted fraction and evaporate it to dryness using rotary vacuum evaporator. Add 1 ml of methanol: water mixture (80:20) (for HPLC) or 1 ml acetone (for GLC analysis), and dissolve the dried mass. Inject this solution in to the HPLC/ GLC units, as the case may be, for final analysis.

NOTE — Before injecting into HPLC system, the sample should be passed through microfilters.

7 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

HPLC unit equipped with UV detector. (For monocrotophos, anilofos, phosalone, temephos and chlorpyrifos.)

The operating parameters suggested may be varied according to the available facilities provided standardization is done.

Column	:	RP - 18 (25 cm × 0.46 cm i.d.) 5 µ
Mobile phase	:	Methanol : Water (80:20)
Detector	:	UV - 225 nm
Temperature	:	Ambient
Flow rate	:	1.2 ml/min
Volume injected	:	20 µl using fixed loop

Working standard solutions of organophosphorus pesticides (in mobile phase) will be given as below:

Monocrotophos	:	0.5 µg/ml
Anilofos	:	2.5 µg/ml
Phosalone	:	5 µg/ml
Temephos	:	5 µg/ml
Chlorpyrifos	:	5 µg/ml

Adjust the volume of the cleaned-up sample solution by dilution with mobile phase to achieve on-scale peaks.

Inject the sample and identify and quantify the peaks by injecting standard solutions of organophosphorus pesticides and by comparing the retention time and area of the sample peak with those of the reference standard (External standardization).

Component	*Retention time*/min	*Recovery %*
Monocrotophos	1.5	84-89
Anilofos	6.2	86-90
Phosalone	7.5	88-92
Temephos	15.0	84-88
Chlorpyrifos	18.1	89-91

8 GAS LIQUID CHROMATOGRAPHY (GLC)

GLC equipped with TID (for Isazophos and Triazophos). The operating parameters suggested may be varied according to the available facilities, provided standardization is done.

Column	:	5% SE-30, Chromosorb WHP (100-120 mesh) 2 m × 1/8" stainless steel column
Oven	:	200°C
Injector	:	230°C
Detector	:	260°C
Nitrogen	:	40 ml/min
Hydrogen	:	5 ml/min

Oxygen : 200 ml/min
Volume injected : 1.0 µl

Working standard solutions of
Isazophos : 0.5 µg/ml (in acetone)
Triazophos : 0.5 µg/ml (in acetone)

Adjust the volume of cleaned up sample solution by dilution with acetone to achieve on-scale peaks. Inject the sample and identify and quantify the peaks by injecting standard solutions of Isazophos and Triazophos and by comparing the retention time and area of the sample peak with that of the reference standard (External standardisation).

Component	*Retention time*	*Recovery %*
Triazophos	5.4 min	92-96
Isazophos	7.3 min	91-94

9 CALCULATIONS

$$\text{Organophosphorus pesticide residue in µg/g (individual pesticide)} = \frac{A_1 \times M_1 \times V_1 \times 100}{A_2 \times V_2 \times M_2 \times f}$$

where

A_1 = peak area of the component of interest in the sample;

A_2 = peak area of the standard;

M_1 = Mass of the standard injected in µg;

M_2 = Mass of the sample taken for analysis in gms;

V_1 = Final volume of sample solution after clean-up in ml;

V_2 = Volume of sample injected, in 5 µl; and

f = Recovery factor = Percentage recovery

10 The methods have the following limits of detection (in micrograms) per gram of the sample analysed:

Monocrotophos : 0.01
Anilofos : 0.05
Phosalone : 0.05
Chlorpyrifos : 0.1
Isazophos : 0.01
Triazophos : 0.01
Temephos : 0.01

Bureau of Indian Standards

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Review of Indian Standards

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