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METHODS FOR DETERMINATION OF CARBOFURAN RESIDUES IN CROPS, SOIL AND WATER

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MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
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METHODS FOR DETERMINATION OF CARBOFURAN RESIDUES IN CROPS, SOIL AND WATER

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Indian Standard

METHODS FOR DETERMINATION OF CARBOFURAN RESIDUES IN CROPS, SOIL AND WATER

0. FOREWORD

0.1 This Indian Standard was adopted by the Indian Standards Institution on 31 August 1984, after the draft finalized by the Pesticides Residue Analysis Sectional Committee had been approved by the Agricultural and Food Products Division Council.

0.2 Carbofuran formulations are extensively used in agriculture for the control of many insect pests. Frequent and increased use of carbofuran formulations often result in harmful effects due to toxic nature of residues. Careful assessment of residues is, therefore, an important step in safeguarding human health and in the establishment of sound regulatory policy.

0.3 This standard will enable the health authorities and others engaged in field to follow uniform test procedure for the estimation of carbofuran residues in crops, soil and water.

0.4 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*.

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1. SCOPE

1.1 This standard prescribes spectrophotometric and gas chromatographic methods for the determination of carbofuran (2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl methyl carbamate), 3 hydroxy carbofuran and 3 keto-carbofuran residues in crops, soil and water.

1.1.1 The spectrophotometric method may be adopted as a limit test for routine purposes and is generally applicable at 0·1 μg/g (0·1 ppm) residue level, whereas, the gas chromatographic method shall be the reference method with the limit of detection of 0·02 μg/g (0·02 ppm).

*Rules for rounding off numerical values (*revised*).

1.1.2 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 carbofuran residues.

2. QUALITY OF REAGENTS

2.1 Unless specified otherwise, pure chemicals and distilled water (*see* IS : 1070-1977*) shall be employed in tests.

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

3. SAMPLING

3.1 The representative samples for the purpose of estimating carbofuran residues in crops, soil and water shall be in accordance with the sampling procedures as prescribed in the relevant Indian Standards, wherever available.

4. PREPARATION OF SAMPLES

4.1 Apparatus

4.1.1 *Buchner Funnel*

4.1.2 *Heating Mantle*

4.1.3 *Round Bottom Boiling Flask* — 1 000 ml capacity with B-24 ground glass joint and an attached water cooled (*Leibig*) condenser.

4.1.4 *Chromatographic Column* — 40 cm long, 2 cm inner diameter.

4.1.5 *Test Tubes* — 25 mm × 190 mm with glass stoppers connected by B-24 ground glass joint.

4.1.6 *Kudrena Danish Evaporative Concentrator*

4.1.7 *Waterbath*

4.1.8 *Separatory Funnel* — 2 litre capacity.

4.2 Reagents

4.2.1 *Hydrochloric Acid* — 0·25 N

*Specification for water for general laboratory use (*second revision*).

4.2.2 *Methylene Chloride* — Analytical reagent grade, glass redistilled.

4.2.3 *Sodium Sulphate* — Anhydrous.

4.2.4 *Alumina* — Column chromatography grade (acidic).

4.2.5 *Silica Gel* — Column chromatography grade.

4.2.6 *Sodium Lauryl Sulphate*

4.2.7 *Diethyl Ether*

4.2.8 *Propylene Glycol*

4.2.9 *Coagulating Solution* — Prepared by dissolving 1 g ammonium chloride in 400 ml of water containing 2 ml of orthophosphoric acid.

4.3 Preparation of Samples — Samples of fruits, vegetables, forages, straw, etc, are chopped finely and mixed well. Samples of grain are ground in a suitable grinder.

4.4 Extraction

4.4.1 *Plant Materials and Soil* — Place 50 g of finely chopped plant, fruit or vegetable, finely ground grain sample, or soil sample in a 1 000 ml round bottom flask and add 600 ml of 0·25 N hydrochloric acid. Connect the round bottom flask to a *Leibig* condenser and reflux the mixture on a heating mantle for one hour, swirling the flask by hand occasionally. After this period, disconnect the condenser and filter the hot sample through glass wool into a 1 000 ml Erlenmeyer flask. Wash the condenser, flask and the glass wool with an additional 300 ml of hot 0·25 N hydrochloric acid. Pool the filtrate and washings and cool the extract in a refrigerator for one hour. Transfer the contents to a 2 litre separatory funnel, add about 0·25 g of sodium lauryl sulphate and mix well. Extract this aqueous phase with three 200 ml portions of the redistilled methylene chloride, passing the methylene chloride solution through a layer of anhydrous sodium sulphate, kept over a funnel with a glass wool pad. Combine the methylene chloride extracts and transfer to a Kudrena Danish evaporative concentrator. Concentrate the extract on a waterbath to about 30 ml.

4.4.2 *Water Samples* — Place an appropriate volume of the water sample (150 to 250 ml) in a 500 ml separatory funnel and extract thrice with 100 ml portions of redistilled methylene chloride, passing the extract through a layer of anhydrous sodium sulphate. Transfer the dried extract to a Kudrena Danish evaporative concentrator and evaporate the solvent on a waterbath to about 30 ml.

4.5 Clean Up

4.5.1 Prepare a chromatographic column in the following order in distinct layers — A layer of glass wool, one gram of anhydrous sodium

sulphate, 10 g of activated alumina, 10 g of silica gel and 2 g of anhydrous sodium sulphate using methylene chloride as the solvent. Wash the column with 100 ml of methylene chloride. Do not allow the solvent to fall to level below the upper layer of the adsorbent packing.

4.5.2 Pour the concentrated extract obtained as in **4.4** slowly into the column, followed by three washings with 10 ml portions of methylene chloride and finally with 50 ml of methylene chloride. When the last liquid has percolated into the column, add 100 ml of diethyl ether and dichloromethane mixture (2:1) and collect the eluate at the rate of 2 ml per minute. Add 3 drops of propylene glycol, and evaporate the solvent almost to dryness in Kudrena Danish evaporative concentrator.

4.5.3 This additional clean-up step is required when the spectrophotometric method is adopted for its determination. Dissolve the residue obtained as in **4.5.2** in 2 ml of acetone and transfer to a glass stoppered 50 ml test tube. Wash the evaporator twice with 2 ml portions of acetone and transfer the washings to the test tube. Add 7 ml of coagulating solution, shake, allow to stand for 10 minutes, with occasional shaking and filter through Whatman No. 42 or equivalent filter paper tube and proceed for determination as given in **5.4.**

5. SPECTROPHOTOMETRIC METHOD

5.1 Principle — The method is based on alkaline hydrolysis of carbofuran and spectrophotometric determination of the resulting phenol with *p*-nitrobenzene diazonium fluoroborate as a chromogenic reagent. The intensity of the complex formed is measured at 550 nm.

5.2 Apparatus

5.2.1 *Spectrophotometer*

5.2.2 *Ice Bath*

5.3 Reagents

5.3.1 *Methanolic Potassium Hydroxide Solution* — 1·5 N.

5.3.2 *Chromogenic Reagent* — Dissolve 25 mg of *p*-nitrobenzene diazonium fluoroborate in 25 ml methanol and mix with 2 ml of glacial acetic acid.

5.3.3 *Standard Carbofuran Solution* — Dissolve 50 mg pure carbofuran in acetone and make up to 100 ml in a volumetric flask. This solution has a concentration of 500 µg/ml. Pipette 4 ml of this solution to a second 100 ml volumetric flask and make up to the mark. This working solution has a concentration of 20 µg/ml.

5.4 Estimation of Carbofuran Residues — Place the test tubes containing 6·5 ml of the cleaned up solution (*see* 4.5.3) in an ice bath, add 2 ml of methanolic potassium hydroxide solution and continue the determination as in standard curve preparation.

5.4.1 *Preparation of the Standard Curve* — Pipette 0, 0·5, 1·0, 2·0, 3·0, 4·0, 5·0 and 6·0 ml of the working standard (20 µg/ml) solution of carbofuran into a series of glass stoppered test tubes and adjust the final volume in all the tubes to 6 ml. Pipette 7 ml of the coagulating solution, allow to stand for 10 minutes with occasional shaking. Pipette 6·5 ml aliquot from each test tube to a second glass stoppered test tube, representing 0, 5, 10, 20, 30, 40, 50 and 60 µg of carbofuran. Place the tubes in an ice bath. Add 2 ml of methanolic potash solution, mix and allow to stand for 5 minutes. Add 1 ml of cold chromogenic reagent and mix well. Read the absorbance of this solution at 550 nm after 2 minutes, against a reagent blank. Prepare the standard curve by plotting absorbance against micrograms of carbofuran.

5.5 Expression of Results — Report carbofuran residues in the samples by using the standard curve, in terms of µg/g (ppm).

6. GAS CHROMATOGRAPHIC METHOD

6.1 Principle — The carbofuran residue in the extracts after clean up is hydrolysed under alkaline conditions to its phenol and is derivatized to its 2, 4-dinitrophenyl ether using 1-fluoro-2, 4-dinitrobenzene. The derivative is extracted in *n*-hexane and estimated by gas chromatographic using either an electron capture or thermoionic detection system.

6.2 Apparatus

6.2.1 *Gas Chromatograph* — A gas chromatograph equipped with either a thermoionic or an electron capture detector is operated under the following suggested parameters. These parameters may be varied as per available facilities provided standardization is done.

Column	Glass, 60 cm long and 4 mm i.d., packed with either 5 percent *OV* — 210 or *OV* 101 on 60-80 mesh gas chrome Q; or 12·5 percent *SE* — 30 on 80-100 mesh chromosorb W (HP)
Column temperature	230°C
Injection port temperature	245°C
Detector temperature	280°C
Gas flow rates	Nitrogen 60 ml/minute. Hydrogen 15 to 18 ml/minute. Air 150 ml/minute
Recorder chart speed	1 cm/minute

6.3 Reagents

6.3.1 *Phosphate Buffer of* pH 11·0 — Dissolve 25·0 g of Na_2HPO_4 in 2 480 ml of distilled water and add 20 ml of 1 *M* NaOH solution. Mix well.

6.3.2 *Reactant* — Dissolve 1·0 g of 1-Fluoro-2, 4-dinitrobenzene (FDNB) in 100 ml of acetone.

6.4 Procedure

6.4.1 *Derivative Formation* — Dissolve the residue obtained in **4.5.2** in 3 ml of the reactant solution (*see* **6.3.2**) and transfer quantitatively to stoppered 25 × 190 mm test tubes. Add 15 ml of the buffer solution, mix well and keep in the water bath maintained at 50°C for 30 minutes. Cool and transfer the mixture to a 60 ml separatory funnel. Extract the solution twice with exactly 10 ml portions of *n*-hexane, collecting the organic layer. Pool the hexane extracts.

6.4.2 *Estimation* — Inject 5 µl of the hexane extract of the derivative into the gas chromatograph. Identify the peak for carbofuran based on its retention time and measure the peak area. The content of carbofuran is determined by comparing the response for a known standard of similar concentration.

6.5 Calculations

$$\text{Carbofuran residue, } \mu g/g \text{ (ppm)} = \frac{As}{Astd} \times \frac{M}{M_1} \times \frac{V}{V_1} \times f$$

where

As = peak area of sample;

M = mass, in µg, of standard injected;

V = final volume of sample in ml;

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

$Astd$ = peak area of the standard;

M_1 = mass, in g, of the sample; and

V_1 = µl of sample injected.

NOTE – Percent mean recovery is determined by taking intreated control sample to which known amount of carbofuran is added and analysed as described above.

AMENDMENT NO. 1 NOVEMBER 1995
TO
IS 11020 : 1984 METHODS FOR DETERMINATION OF CARBOFURAN RESIDUES IN CROPS, SOIL AND WATER

(*Page* 4, *clause* 2.1) — Substitute 'IS : 1070 - 1992*' *for* 'IS : 1070 - 1977*'.

(*Page* 4, *foot-note with* '*' *mark*) — Substitute 'Reagent grade water (*third revision*)' for the existing.

(FAD 34)

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