IS 13829 : 1993

(Reaffirmed 1998)

भारतीय मानक

कीटनाशी — कृषि और खाद्य पदार्थों, मिट्टी एवं पानी में अवशिष्ट ज्ञात करने की पद्धति — एट्राजिन एवं सिमाजिन

Indian Standard

PESTICIDE — METHOD FOR DETERMINATION OF RESIDUES IN AGRICULTURAL AND FOOD COMMODITIES, SOIL AND WATER — ATRAZINE AND SIMAZINE

UDC 664 : 543 [632·95·028 ATR]

BUREAU OF INDIAN STANDARDS
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NEW DELHI 110002

October 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 Agriculture Division Council.

Both, atrazine (2-chloro-4-ethylamino-6-isopropylamino-5-triazine) and simazine (2-chloro-4, 6-bis-ethylamino-s-triazine) are used as herbicides in agriculture for the control of weeds. Assessment of their residues in food commodities is therefore an important step in safeguarding human health and establishment of regulatory policy.

This standard will enable the health authorities and others engaged in the field to follow uniform test procedures for the estimation of residues of atrazine and simazine in various commodities.

In the preparation of this standard, due consideration has been given to the maximum limits of atrazine and simazine 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 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 — METHOD FOR DETERMINATION OF RESIDUES IN AGRICULTURAL AND FOOD COMMODITIES, SOIL AND WATER — ATRAZINE AND SIMAZINE

1 SCOPE

1.1 This standard describes gas chromatographic (GLC) and high performance liquid chromatographic (HPLC) methods for determination of atrazine and simazine residues in food commodities.

1.2 The limit of determination of both the compounds is 0·05 μg/g by the PHLC method, whereas it is 0·01 μg/g by the GLC method.

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 atrazine and simazine.

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. 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.

4 SAMPLING

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

5 APPARATUS

5.1 Waring Blender

5.2 Laboratory Shaker (Rotary Action)

5.3 Chromatographic Column, 25 cm long × 2 cm i.d.

5.4 Rotary Vacuum Evaporator

5.5 Air Blower

5.6 Water Bath

5.7 Gas Chromatograph

Equipped with a nitrogen specific 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 length, 4 mm internal diameter packed with 5 percent OV-101 on Gaschrom Q (60-80) mesh		
Temperatures	: Column oven	:	170°C
	Injector	:	200°C
	Detector	:	210°C
Carrier gas (nitrogen) flow rate	: 30 ml/min		
Retention time	: Simazine	:	3 minutes approximately
	Atrazine	:	3·5 minutes approximately

5.8 Microlitre Syringe — 10 μl capacity.

5.9 High Performance Liquid Chromatograph

Equipped with a variable wave length ultraviolet detector, operating under the following suggested parameters. These parameters may be varied according to the available facilities provided standardization is:

Column	: Zorbax ODS or Partisil ODS 25 cm length and 4·6 mm internal diameter		
Mobile phase	: Methanol		
Flow rate	: 1 ml/min		
Wave length	: 230 nm		
Absorbance range	: 0·02		
Retention time	: Simazine	:	6 minutes approximately
	Atrazine	:	8 minutes approximately

6 REAGENTS

6.1 Chloroform — HPLC grade

6.2 Petroleum Ether — boiling range 40-60°C.

6.3 Ethyl Ether — 5 percent (*v/v*) in carbon tetrachloride.

6.4 Carbon Tetrachloride — HPLC grade.

6.5 Acetonitrile — HPLC grade.

6.6 Methanol — HPLC grade.

6.7 Sodium Acetate Buffer Solution

Mix equal volumes of 2 N acetic acid and 1 N sodium hydroxide solution.

6.8 Reactivated Basic Alumina

Mix 90 g basic alumina with 10 ml water thoroughly and allow to stand overnight.

6.9 Acetic Acid — 2 N.

6.10 Sodium Hydroxide Solution — 1 N.

6.11 Sodium Sulphate — Anhydrous.

6.12 Ethyl Acetate — AR grade.

6.13 Reference Standard Atrazine — of known purity.

6.14 Reference Standard Simazine — of known purity.

7 EXTRACTION

7.1 Grain, Straw, Hay (Low Moisture) and Soil

Transfer a suitable quantity (100-200 g) finely ground sample into a waring blender, add 100-ml chloroform and homogenize for 5 minutes. Transfer the contents quantitatively to a stoppered 1 000-ml conical flask, add 400-ml chloroform and shake the slurry vigorously on a rotary-action laboratory shaker for one hour. Filter the extract by decanting, through a layer of anhydrous sodium sulphate mounted on a Whatman No. 1 or equivalent filter paper and a funnel. Note the exact volume of the filtrate obtained. Evaporate an aliquot of the extract, equivalent to 50 g of the crop sample, carefully to dryness on a water bath in a 250-ml beaker, with the help of a gentle stream of air.

7.2 Fruit and Vegetables

Transfer a suitable quantity (100 g) of the finely chopped fruit or vegetable sample into a waring blender along with about 100 g anhydrous sodium sulphate and 100 ml of chloroform. Blend the mixture for 5 minutes and continue further extraction as described in **7.1**.

7.3 Fatty Crops, such as Oilseeds and Nuts

Follow the extraction procedure described in **7.1**. Evaporate chloroform completely on the water bath with the help of stream of air and dissolve the residue in 50 ml petroleum ether. Transfer the solution to a 250-ml separatory funnel and wash the beaker twice with 20 ml portions of petroleum ether trasferring the washings into the separatory funnel. Extract the petroleum ether solution in the separatory funnel thrice with 25-ml portions of acetonitrile. Pool the acetonitrile extract, and transfer to a second 250-ml separatory funnel and wash with 50-ml petroleum ether. Discard the petroleum ether layer. Transfer the acetonitrile solution quantitatively, directly to the round bottom flask of the rotary vacuum evaporator and completely evaporate off the acentonitrile under slightly reduced pressure and with a water bath at 50°C.

7.4 Meat and Egg

Transfer a suitable quantity (100-200 g) of the sample into a waring blender along with an equal quantity of anhydrous sodium sulphate and blend the contents for 5 minutes. Add 100 ml of chloroform, and homogenize for further 5 minutes. Continue the extraction as per the procedure described in **7.1** and then continue as in **7.3**.

7.5 Milk

Transfer 200 ml of the milk sample into a 600-ml beaker, and 200-ml methanol and mix thoroughly by stirring with a glass rod. Introduce, with stirring, 20-ml sodium acetate buffer solution. Mix well and keep in the ice bath for 30 minutes. Filter the mixture through a fluted filter paper and rinse the filter with a jet of water. Collect the filtrate and water washings in a 600-ml beaker. Transfer the filtrate to a 1 000-ml separatory funnel and extract thrice with 60-ml portions of chloroform. Emulsions can be avoided by the addition of a 2-ml saturated solution of sodium chloride. Pool the chloroform extracts in a 250-ml beaker and evaporate to dryness on a water bath with the help of a stream of air.

7.6 Water

Transfer 500 ml of the water sample into a 1 000-ml separatory funnel and extract the aqueous layer thrice with 60-ml portions of chloroform. Pool the chloroform extracts in a 250-ml beaker and evaporate the contents to dryness on a water bath with the help of a stream of air.

8 CLEAN UP

Add 25 g reactivated basic alumina to the chromatographic column, tap gently to eliminate channeling and to achieve uniform packing. Dissolve the extracted sample residue (*see* 7) in 10-ml carbon tetrachloride, transfer on to the column and allow to penetrate into the alumina. Wash the beaker with 10 ml of carbon tetrachloride and transfer to the column allowing to penetrate as before. This operation shall be further repeated with another 5 ml of tetrachloride. When the solvent has just penetrated into the column, add 80 ml of carbon tetrachloride and allow to pass through the alumina layer. When the last drop of the carbon tetrachloride has drained down, place a clean 250-ml beaker as receiver, and add 100 ml of 5 percent ethyl ether in carbon tetrachloride collecting the complete eluate in the beaker. Evaporate the contents of the beaker to dryness on a water bath with a stream of air.

9 GAS CHROMATOGRAPHIC METHOD

9.1 Principle

The residue of atrazine or simazine extracted from the sample after clean up is dissolved in ethyl acetate and estimated gas chromatographically in an instrument equipped with a nitrogen specific detector. The content of atrazine or simazine in the sample is determined by comparing the response with the response of a known standard of similar concentration.

9.2 Procedure

Dissolve the residue after clean up (*see* 8) in 2-ml ethyl acetate and inject 2 ml of this solution into the gas chromatograph. Identify the peak for atrazine or simazine by the retention time and measure the peak area.

9.3 Calculation

$$\text{Residue of atrazine or simazine} = \frac{A_1 \times V_2 \times V_3 \times C}{A_2 \times V_1 \times M} \times f$$

where

A_1 = peak area of the sample;

V_2 = volume, in μl, of standard atrazine or simazine injected;

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

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

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 atrazine or simazine is added and analyzed as described above.

10 HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC (HPLC) METHOD

10.1 Principle

The residues of atrazine or simazine extracted from the sample after clean up is dissolved in methanol and estimated by HPLC equipped with ultra-violet detector. The content of atrazine or simazine is determined by comparing the response with the response of a known standard of similar concentration.

10.2 Procedure

Dissolve the residue after clean up (*see* 8) in 2-ml methanol and inject 5 μl of this solution into the HPLC instrument. Identify the peak for atrazine or simazine and measure the peak area.

10.3 Calculation

$$\text{Residue of atrazine or simazine } (\mu g/g) = \frac{A_1 \times V_2 \times V_3 \times C}{A_2 \times V_1 \times M} \times f$$

where

A_1 = peak area of the sample;

V_2 = volume, in μl, of standard atrazine or simazine 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 atrazine or simazine is added and analyzed as described above.

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