INTRODUCTION

The Residue Analysis Unit conducted a study to determine tetraconazole residues in soil samples obtained from a field trial carried out by SIPCAM Experimental Service on behalf of ISAGRO S.p.A. at Salerano sul Lambro (LO) in the Northern part of Italy (Lombardia Region, see Appendix).

The formulated product (TETRACONAZOLE) was applied to bare soil (loamy sand type) at a nominal concentration of 120 g/ha (3 L/ha). Soil cores were taken at different sampling times, during 1 year period, from 4th August 1997 to 4th August 1998.

The study was carried out in accordance with protocol 2226 (Enclosure B).

PROCEDURES

STANDARD

Tetraconazole reference standard used in this study was supplied by ISAGRO RICERCA with its certificate of analysis (Enclosure A) and was stored in refrigerator at about +4°C.

The working standard solution used both for the linearity check and as reference in the gas chromatographic analysis, were prepared by dissolving in ethyl acetate an amount of standard, accurately weighed according to the Standard operating procedure (henceforth named SOP) coded A0BIL1, in a 100 mL capacity volumetric flask. Diluted working standard solutions were prepared for linearity check, as external reference standard and also for sample fortification (see below).

The working standard solutions thus prepared were stored at +4°C in glass polyethylene capped flasks.

After each sampling, the weight of the flask was recorded. Immediately before the next sampling, the flask was weighed again: if a difference occurred in the second decimal figure, the solution was newly prepared.

SAMPLE FORTIFICATION

Working standard solutions at different concentrations were prepared by diluting the ethyl acetate solution with acetone.

Known amounts of these acetonic solutions were mixed with an exactly weighed amount of untreated soil (control) in a glass round bottomed flask and acetone was evaporated at reduced pressure (t max 30 °C) to constant weight.

Each fortified sample was assigned a **unique code** by the Residue Analysis Unit, according

to the SOP coded A3CODI.

The fortification volume and the corresponding concentration of each sample with its identification code are shown together with the recovery data.

SAMPLE ORIGIN AND IDENTIFICATION

Frozen soil samples and **Petri dishes** coming from a field trial (code: ISA/1) have been received according to the SOPs A1CARI and A2RIMO by the testing facility in four occasions, and the following NOR (Number of Reception) codes were attributed:

NOR 318 for the 14th January 1998 shipment;

NOR 319 for the 15th January 1998 shipment;

NOR 335 for the 9th July 1998 shipment;

NOR 336 for the 7th August 1998 shipment;

from:

SIPCAM Eperimental Service
c/o SIPCAM S.p.A.
Via Vittorio Veneto, 81
26857 Salerano sul Lambro Lodi (Italy)
Fax:+39-371-71408
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as detailed in the shipping documents in Enclosure C.

Each sample consisted of ten acetate tube cores (5 cm diameter x 30 cm length) for each sampling time.

ANALYTICAL METHOD

1. PRINCIPLE OF THE METHOD

Tetraconazole is extracted from soil by a methyl alcohol:water mixture, followed by dichloromethane liquid/liquid partitioning. Final extracts are purified by column chromatography on alumina. The active ingredient is determined by gas chromatography using an flameless ionization detector, calibrated for nitrogen.

2. REAGENTS

- Analytical RPE grade methyl alcohol (e.g.: Carlo Erba - Milan Italy).
- Analytical RPE grade dichloromethane (e.g.: Carlo Erba - Milan Italy), redistilled in a glass apparatus.
- Analytical RPE grade hexane (e.g.: Carlo Erba - Milan Italy), redistilled in a glass apparatus.
- Analytical RPE grade acetone (e.g.: Carlo Erba - Milan Italy), redistilled in a glass apparatus.
- Analytical RS grade ethyl acetate (e.g.: Rudi Pont - Milan Italy), redistilled in a glass apparatus.
- saturated aqueous solution of analytical grade sodium chloride (e.g.: Carlo Erba - Milan Italy)
- alumina, grade II-III according to Brockman (e.g.: Merck - Milan Italy)
- reference analytical standard of tetraconazole
- anhydrous sodium sulphate analytical RPE grade (e.g.: Carlo Erba - Milan Italy).
- Hydrogen gas for gas chromatography (SIO Novara Italy).
- Helium gas for gas chromatography (SIO Novara Italy).
- Nitrogen gas for gas chromatography (SIO Novara Italy).

3. EQUIPMENT

- Glass filtering unit (Witt apparatus) equipped with a glass sintered Büchner funnel (ϕ 9 cm) and water vacuum pump (Carlo Erba Milan Italy).
- Horizontal shaker for separatory funnel and conical flasks (e.g.: mod.HS501 digital IKA Milan Italy).
- Ultrasonic bath (e.g. mod. B3 Branson Milan Italy).
- Glass fiber paper filter (e.g. GF/C ϕ 9 cm and ϕ 4.7 cm, Whatman Milan Italy).
- Vacuum rotary evaporator (e.g.: LABO ROTA mod S300 Resona Technics Milan

Italy).

- Gas chromatograph (e.g.: Mega Series II GC mod.8530 Carlo Erba Milan Italy) equipped with NPD-80 FL flameless detector and automatic peak integrator (e.g.: DP800 CH2 Carlo Erba Instruments Milan Italy).

- Gas chromatographic semicapillary column (e.g. SPB 5, length 30m, i.d. 0.53 mm, film thickness 1.5 µm - Supelco Milan Italy).

- Gas chromatograph (e.g.: HRGC Carlo Erba, Milan Italy) equipped with ECD detector and automatic peak integrator and elaborator (e.g.: Shimadzu - mod.CR4-A Milan Italy).

- Gas cromatographic semicapillary column (e.g. SPB 1, length 30m, i.d. 0.75 mm, film thickness 1 µm - Supelco Milan Italy).

- 10 µL gas chromatographic syringe (e.g. Hamilton Milan Italy)

- technical balance (e.g. Sartorius mod. LC 820 - Zeiss Milan Italy)

- analytical balance (e.g. Sartorius mod. RC 250S - Zeiss Milan Italy)

- common analytical laboratory glassware and equipment for chemical laboratory.

4. METHOD

The method works out through these three steps:

PHASE 1: EXTRACTION
PHASE 2: PURIFICATION
PHASE 3: ANALYSIS

4.1 PHASE 1: EXTRACTION

Shake for 30 min at 200 strokes per min, a 500 mL glass conical flask, 100 g of substrate with 100 mL of a methyl alcohol:water (9:1 v/v) mixture.

Filter throught glass fiber paper (ϕ 9 cm) under vacuum.

Repeat the extraction procedure two more times.

Rinse with 20 mL of the same mixture all the glass equipment and collect all the hydroalcoholic extracts in a 1 liter round bottomed flask. Evaporate under vacuum

(t max 35 °C) all the organic solvent at constant weight.

Add distilled water to a final volume of 100 mL and transfer the aqueous extract in a 250 mL glass separatory funnel, equipped with a teflon stopcock. Wash the 1 liter round bottomed flask twice with 50 mL dichloromethane each time.

Keep for 15 min in the horizontal shaker at 200 strokes per min, then left to settle in vertical position for about 1 hour to obtain a complete separation of the organic layer from the upper aqueous one.

Filter the lower organic phase through 30 g anhydrous sodium sulphate, previously washed with freshly distilled dichloromethane.

Collect the filtrate in a round bottomed flask of proper size and evaporate the solvent under reduced pressure in a rotavapor (max temp. 30 °C).

4.2 PHASE 2: PURIFICATION

4.2.1 Column preparation

Pour 15 mL mixture n-hexane : acetone (50:50 v:v) into the chromatographic tube. Slowly add 20 g alumina (free from air bubbles). Allow to settle then add 5 g anhydrous sodium sulphate. Drain the solvent and wash with further 50 mL of the same mixture, at a flow rate of 1.5 mL/min until the liquid cover the top of the filling.

4.2.2 Extract loading

Dissolve the residue obtained after solvent evaporation (see § 4.1) with 2 mL of the same n-hexane:acetone mixture, using the sonicating bath, if necessary.

Transfer quantitatively into the column so prepared, leaving the liquid to penetrate into the filling.

Wash twice the flask with 1 mL of the same solvent mixture and top to the column.

4.2.3 Chromatographic elution

Eluate with the same solvent mixture: discard the first 30 mL fraction and collect the following 70 mL fraction (see Important points).

Evaporate the solvent to dryness as above mentioned. Dissolve the residue in a suitable volume of ethyl acetate (from 1 up to 50 mL, according to the hypothetical concentration of tetraconazole).

4.3 PHASE 3: ANALYSIS

Dissolve the residue in an appropriate volume of ethyl acetate and analyse by gas chromatography using a nitrogen phosphorous detector set for nitrogen (GC/NPD).

4.3.1 Confirmatory method

A different confirmatory method has been set up using a different detector (ECD) and a different column (semicapillary, SPB 1).

4.3.2 Operative conditions

The operative conditions used with the different columns and gas chromatographic apparatus employed are shown in the gas chromatographic section.

The SPB - 5 semicapillary column set on the HRGC MEGA series 8530 with flameless detector (FL NPD 80) was chosen for this study.

5. CALCULATIONS.

Calculation of tetraconazole is accomplished by the external standard method, on at least duplicate injections.

Exactly measured volumes of a tetraconazole reference standard solution at known concentration (C_{std}) are injected just before and after sample analysis.

Peak height of treated sample (H_{smp}), of blank (untreated) sample (H_{bkg}), and of standard (H_{std}) are recorded.

The concentration of tetraconazole present in the original sampled substrate is then calculated by applying the formula below:

$$R = \frac{(H_{smp} - H_{bkg}) * C_{std} * V_F}{H_{std} * W}$$

where:

R residue (in mg/kg)

H_{smp} unknown treated sample peak height (arbitrary units: datum is the mean of at least

duplicate injections)

H_{bkg} unknown blank (untreated) sample peak height (arbitrary units: datum is the mean of at least duplicate injections)

C_{std} reference standard concentration (mg/L)

V_F final purified volume (mL)

H_{std} reference standard peak height (arbitrary units: datum is the mean of at least duplicate injections)

W amount of the sample (e.g. 100 g)

The subtraction of H_{bkg} from H_{smp} in the equation above shown, is performed only if H_{bkg} is higher than 10% of H_{smp}.

The time necessary to perform a complete analysis (including data elaboration) is about 4 hours/man.

6. IMPORTANT POINTS

The recovery factor of the method may be strongly affected by interfering substances; as a consequence it is necessary to perform always recovery tests at different fortification levels.

For the same reason it could be necessary to modify either the ratio of the solvent mixture used for the clean-up step or the operative conditions of analysis.

Moreover, different batches of alumina may require different elution conditions: for this reason it is necessary to check the recovery of tetraconazole from the purification column for every new lot of the stationary phase.

Beware of tumultuous boiling at the beginning of solvent evaporation.

Sonicate solutions whenever rinsing and washing operations are carried out.

tetraconazole

Fungicid

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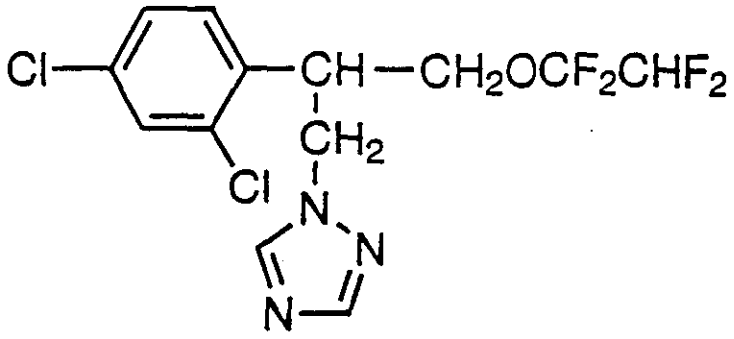

NOMENCLATURE

Common name tetraconazole (BSI, draft E-ISO).

IUPAC name (*RS*)-2-(2,4-dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propyl 1,1,2,2-tetrafluoroethyl ether.

C.A. name (±)-1-[2-(2,4-dichlorophenyl)-3-(1,1,2,2-tetrafluoroethoxy)propyl]-1*H*-1,2,4-triazole. **CAS RN** *[112281-77-3]* unstated stereochemistry

Development code M 14 360 (Agrimont).