APPENDIX E

DUPHAR B.V. WEESP
Analytical Development Dept.
The Netherlands
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LABORATORY INSTRUCTIONS
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GAS CHROMATOGRAPHIC DETERMINATION OF DICHLOBENIL AND 2,6-DICHLOROBENZAMIDE IN SOIL

1. PRINCIPE

Soil is extracted with a mixture of acetone/petroleum ether (1:1). The extract is filtered and after addition of water, dichlobenil is extracted with petroleum ether, subsequently 2,6-dichlorobenzamide is extracted with ethyl acetate. An aliquot of both extracts is purified on alumina cartridges and determined by capillary gas chromatography and electron capture detection.

2. REQUISITES

Reagents

- acetone (Baker 8002
- water for injections
- ethanol, pharmaceutical quality
- sodiumchloride-solution, saturated (Brocacef NA734)
- petroleum ether (b.p. 40-65°C), spectrograde
- acetone/petroleum ether (b.p. 40-65°C) (1+9)
- acetone/petroleum ether (b.p. 40-65°C) (2+98)
- ethanol/petroleum ether (b.p. 40-65°C) (15+85)
- ethyl acetate (Baker 9260-3)
- methanol, spectograde
- NH_4Cl (Baker 0018)
- Na_2SO_4, anhydrous (Baker 1775)
- alumina cartridge, neutral, 1000 mg (Baker 7214-07)
- helium, pure (carrier gas)
- nitrogen, pure (make-up gas)
- 2,6-dichlorobenzamide ARS
- dichlobenil ARS

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Equipment

- Ultra Turrax (e.g. IKA T50 with G45G)
- grinding beakers
- ultrasonic bath (e.g. Branson)
- rotavapor
- gas chromatograph for capillary gas chromatography, equipped with an ^{63}Ni electron capture detector and suitable for cold-on-column injection.
- column for capillary gas chromatography, fused silica (length 30 m, i.d. 0.32 mm) coated with 0.25 μm DB-17 and coupled with an uncoated deactivated retention gap (length 30 cm, i.d. 0.53 mm) for dichlobenil and coupled with an retention gap coated with 0.05μm of OV-225 (CP Sil 43 CB, length 30 cm, i.d. 0.53 mm) for 2,6-dichlorobenzamide.
- syringe, suitable for on-column injection on capillary columns, 3 μl.

3. PROCEDURE

Gaschromatography

3.1 Dichlobenil

Adjust the pressure of the carrier gas to 100 kPa and adjust the flow of the make-up gas to 25 ml/min. Set the temperature of the column oven at 75°C, and the detector temperature at 280°C. Install an oven temperature programme with a 30°C/min rate till 180°C, starting at injection. Keep at 180°C for 3 min., program the temperature to increase by 30°C/min up to 225°C. Set the secundaire cooling at 10 seconds after injection.

3.2 2,6-Dichlorobenzamide

Adjust the pressure of the carrier gas to 125 kPa and adjust the flow of the make-up gas to 25 ml/min. Set the temperature of the column oven at 70°C and the detector at 280°C. Install an oven temperature programme with a 30°C/min rate till 180°C, starting 1 min. after injection. Set the secundaire cooling at 10 seconds after injection.

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Calibration line

3.3 Prepare solutions containing about 10 mg of dichlobenil and 2,6-dichlorobenzamide (accurately weighed) per 100.00 ml of methanol (=solution A). Dilute these solutions ten times with acetone (=solution B). Dilute these solutions again ten times with acetone (=solution C). Prepare by further dilution with petroleum ether (for dichlobenil) and ethyl acetate (for 2,6-dichlorobenzamide), seven solutions containing respectively 0, 0.01, 0.03, 0.05, 0.08, 0.1, 0.2 µg per ml of dichlobenil (petroleum ether solutions) and 2,6-dichlorobenzamide (ethyl acetate solutions).

3.4 Inject 1.0 µl of each solution and chromatograph under conditions mentioned in sections 3.1 (for dichlobenil) and 3.2. (for 2,6-dichlorobenzamide).

3.5 Determine the height of each dichlobenil and 2,6-dichlorobenzamide peak.

3.6 Plot the peak heights found against the corresponding concentrations in the injected solutions in µg/ml. Draw a line through the points. This is the calibration curve.

Extraction

3.7 Homogenise the soil sample and weigh about 50 g of soil.

3.8 Transfer the analytical sample of 50 g into the grinding beaker, add 150 ml of acetone/petroleum ether (1:1) and 17 ml 0,2%-NH_4CL-solution and grind for 5 minutes with an Ultra Turrax.

3.9 Filter through a Büchner funnel into a suction flask and wash with 30 ml of acetone/petroleum ether (1:1).

3.10 Transfer the extract into a separating funnel, add 300 ml water, 10 ml saturated NaCl-solution and shake for 1 minute. Allow the layers to separate and collect the petroleum ether-layer through a fluted filter, containing anhydrous Na_2SO_4, into a round bottom flask.

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3.11 Add 100 ml of petroleum ether to the water layer and shake for 1 minute. Allow the layers to separate and collect the petroleum ether through the same fluted filter containing anhydrous Na_2SO_4.

3.12 Repeat 3.11 two more times and combine the petroleum ether extracts. Keep the water layer.

3.13 Evaporate at ambient temperature till a volume of 30 ml remains and transfer this solution into a 50 ml volumetric flask and make-up to volume with petroleum ether.
This is the dichlobenil extract.

3.14 Add 100 ml of ethyl acetate to the water layer of section 3.12 and shake for 1 minute. Allow the layers to separate and collect the ethyl acetate through a fluted filter containing anhydrous Na_2SO_4, into another round bottom flask.

3.15 Repeat 3.14 two more times and combine the ethyl acetate layers extracts.

3.16 Evaporate to dryness and dissolve the residue in 10.00 ml acetone/petroleum ether (1:1).
This is the 2,6-dichlorobenzamide extract.

Clean-up

Dichlobenil

3.17 Condition, with the aid of vacuum, an alumina cartridge with 5 ml of petroleum ether.

3.18 Bring 5.0 ml of the dichlobenil extract onto the cartridge.

3.19 Wash with 1 ml of petroleum ether.

3.20 Elute with 8 ml of 2% acetone/petroleum ether solution into a 10 ml volumetric flask and make-up to volume with 2% acetone in petroleum ether.
This is the dichlobenil sample solution.

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2,6-dichlorobenzamide

3.21 Condition, with the aid of vacuum, an alumina cartridge with 5 ml of petroleum ether.

3.22 Bring 5.0 ml of the 2,6-dichlorobenzamide extract onto the cartridge.

3.23 Wash two times with 5 ml portions of acetone/petroleum ether (1+9).

3.24 Elute with 5 ml of ethanol/petroleum ether (15+85).

3.25 Evaporate the solvents (40°C) and dissolve the residue in 5.0 ml of ethyl acetate.
This is the 2,6-dichlorobenzamide sample solution.

Sample chromatography

3.26 Inject 1.0 µl of the final dichlobenil sample solutions (3.20) under conditions mentioned in section 3.1.

3.27 Determine the height of the dichlobenil peaks. Read off from the calibration curve the concentration of dichlobenil wich corresponds to the peak heights.

3.28 Repeat sections 3.26 and 3.27 for 2,6-dichlorobenzamide, using sample solution of section 3.25 (GC conditions in section 3.2)

4. CALCULATION

The dichlobenil and 2,6-dichlorobenzamide content is:

$$\frac{p \times v}{w} = \text{mg/kg}$$

p = concentration read off from the calibration line in µg/ml
v = final volume of the sample in ml (for dichlobenil 100, for 2,6-dichlorobenzamide 10)
w = weight of the sample in g = 50.

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5. REMARKS

5.1 The retention time of dichlobenil is approx. 5 minutes. For 2,6-dichlorobenzamide the retention time is approx. 11 minutes.

5.2 Since dichlobenil and 2,6-dichlorobenzamide are compounds of quite different polarity, different solvents and different GC conditions were used.

5.3 Retention gaps, as used for both determinations, could get effected by involatile sample constituents after a series of sample injections. This is indicated by a decreasing peak-shape (more tailing). Replacing the retention gap will restore the initial chromatographic performance.

5.4 Calibration curves will, in general, be best fitted by a quadratic logarithmic expression.