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HLA 6012-271

14/DEC/1988

RES 026

LIQUID CHROMATOGRAPHIC DETERMINATION OF RESIDUES OF DIFLUBENZURON IN SOIL

1. PRINCIPLE

The diflubenzuron is extracted from the soil by refluxing with acetonitrile/water. After filtration of the extract, an aliquot is extracted with hexane. If necessary the extract is "cleaned" by chromatography on florisil. Diflubenzuron is determined by liquid chromatography with UV detection at 254 nm.

2. REQUISITES

Reagents

	Specification
- hexane	LRS 1322 830 75113
- diflubenzuron, ARS	
- acetonitrile (Baker 8143)	
- dioxane (Merck 3132)	
- water for injections	
- ethanol, 100%, pharmaceutical	LRS 1322 830 78104
- acetone (Baker 8002)	LCS 1322 505 39201
- petroleum ether (b.p. 40-65°C), spectrograde	LRS 1322 830 76703
- acetone/petroleum ether (b.p. 40-65°C) (1-9)	
- acetone/petroleum ether (b.p. 40-65°C) (1-4)	
- dichloromethane, residue quality	
- Florisil with 5,5% of water, 60-100 mesh	
- mobile phase (acetonitrile/water/dioxane 45/45/10)	
- column packing, Zorbax CN, 7-8 um (Du Pont de Nemours)	

Apparatus

- liquid chromatograph with UV detection at 254 nm and recorder
- V4A-stainless steel column, 25 cm, int. diam. 4,6 mm, filled with Zorbax C8 (7-8 um) and a Brownlee pre-column (C8)
- ultrasonic bath
- chromatography tubes (length 450 mm, i.d. 12 mm)

3. PROCEDURE

3.1 Fill the column with the column packing using the slurry method.

3.2 Connect the column to the injection valve and the detector. Adjust the flow rate of the mobile phase to 3 ml/min and allow to stabilise at 35°C.

Calibration line

3.3 Weigh out about 10 mg of diflubenzuron accurately into a 100-ml volumetric flask. Dissolve and make up to volume with dioxane. This is "solution A".

Standa

3.4 Pipette 10 ml of "solution A" into a 100-ml volumetric flask. Make up to volume with dioxane. This is "solution B".

3.5 Pipette 0, 2, 4, 6, 8 and 10 ml of "solution B" into 6 100-ml volumetric flasks respectively. Add 10, 8, 6, 4, 2 and 0 ml of dioxane respectively. Adjust to 100 ml using acetonitrile/water (1+1).

3.6 The concentration of diflubenzuron in the 6 solutions is respectively 0, 0.2, 0.4, 0.6, 0.8 and 1 ug/ml ("calib 1 to calib 6")

3.7 Inject 100 ul of "calib 1 to calib 6" and chromatograph; correct for sensisitivity changes by injecting "calib 4" every 3 samples.

3.8 Measure the peak height of the diflubenzuron and plot the peak height against the concentration (in ug/ml of the final solution). This is the calibration line.

Samples

3.9 Reflux 50 g (v g) of sample with 30 ml of water and 170 ml of acetonitrile for 30 minutes.

3.10 After cooling to ambient temperature, filter the extract through a fluted filter.

3.11 Evaporate 100.0 ml of the filtrate to about 15 ml. Transfer the solution to a separating funnel with 85 ml of water and extract three times with 50-ml portions of hexane for 2 minutes.

3.12 Collect the hexane layers in a round bottom flask and evaporate to dryness using 5 ml of 100% ethanol to remove the water.

Clean up

3.13 Fill a chromatography tube with 20 cm of Florisil (= 11.7 g). Close the column with a plug of adsorbent cotton wool and wash with 100 ml of petroleum ether.

3.14 Dissolve the residue (3.12) in 3 ml of dichloromethane and add 25 ml of petroleum ether.

3.15 Transfer the solution with 25 ml of petroleum ether to the column. Chromatograph, if necessary with the aid of air pressure, at the rate of 2 drops/second (= about 5 ml/min). Reject the effluent.

3.16 Wash the column successively with 45 ml of petroleum ether, 3 x 10 ml of acetone/petroleum ether (1 + 9) and 10 ml of acetone/petroleum ether (1 + 4). Reject the effluents. Elute with 2 x 25 ml of acetone/petroleum ether (1 + 4) into a 100-ml round bottom flask.

3.17 Evaporate to dryness in a water bath at 70°C. Dissolve the residue in 1.00 ml of dioxane while vibrating for 10 seconds in an ultrasonic bath.

Add 9.00 ml of acetonitrile/water (1/1).

3.18 Inject 100 µl and chromatograph; correct for sensitivity changes by injecting "calib 4" every 3 samples.

3.19 Measure the height of the diflubenzuron peak. Read off from the calibration line the quantity of diflubenzuron which corresponds to this peak.

4. CALCULATION

The content of diflubenzuron is:

$$\frac{C_e \times f}{v} = \quad \text{ng/kg}$$

C_e = concentration read off from the calibration line, in ug/ml
f = final volume of the sample, in ml = 20
v = weighed quantity of sample, in g = 50

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14/DEC/1988

RES 025

GAS CHROMATOGRAPHIC DETERMINATION OF RESIDUES OF PARACHLOROPHENYL UREA (CPU) IN SOIL

1. PRINCIPLE

The CPU is extracted using ethyl acetate, the extract is evaporated to dryness and dissolved in acetonitrile. The solution is purified by washing with hexane. The extract is further purified by column-chromatography on silica gel.
After derivatisation of the CPU, using heptafluorobutyric anhydride (HFBA), it is chromatographed on a column of 3% Carbowax 20 M and detected with an electron-capture detector. The CPU is determined by comparing the peak height of the sample with that of a derivated CPU standard solution.

2. REQUISITES

Reagents	Specifications
- ethyl acetate (Baker no. 3-9281)	
- hexane, spectrograde	LRS 1322 830 75113
- acetonitrile for chromatography	LRS 1322 830 73734
- acetone (Baker no. 8002)	
- petroleum ether (boiling point 40-65°C), spectrograde	LRS 1322 830 76703
- heptafluorobutyric anhydride, distilled over P_2O_5 (Pierce no. 63163)	
- water for injections	LRS 1322 830 78104
- ethanol 100%, v/v pharmaceutical grade	LCS 1322 505 39201
- CPU, standard solutions in acetonitrile (from 0.01 ug/ml to 0.1 ug/ml)	

- nitrogen pure (carrier and supplementary gas)
- silica gel with 4.4% water (0.063-0.2 mm)
- sodium sulphate, anhydrous
- column packing 2)
- sodium carbonate (Baker no. 0274), saturated solution in water
- absorbent cotton wool 3)

Notes

1) Add 10 g of P_2O_5 granules to 100 ml of HFBA. Reflux for 30 minutes. Distil using a vigreux and a Liebig condenser. Reject the first and the last 10 ml.

2) Prepare the column packing according to LMI 1322 830 74904, sections A.2 (silanising), B.1 (slurry method) and C (drying), using the following data:

stationary phase A: Carbovax 20 M 1%
solvent B : chloroform
support C : Chromosorb W HP 100 - 120 mesh

Silanise a glass column according to LMI 1322 830 74904 section D.

3) Using ether, extract the grease from the cotton wool (24 hours in a Soxhlet apparatus)

Apparatus

- mixer (e.g. Townson and Mercer)
- chromatographic tubes (length 20 cm. int.diam. 12 mm)
- separating funnels with teflon top
- amber-coloured bottles with screw-caps and rubber-laminated teflon discs
- gas chromatograph with electron-capture detector and recorder
- syringe 10 µl (e.g. Hamilton)
- glass, spiralised column (length 100 cm. int. diam. 3 mm) filled with column packing

3. PROCEDURE

3.1 Homogenise the soil.

Extraction

3.2 Add to 50 g (=w g) of sample 25 ml of water, let it stand for 15 minutes.

3.3 Add 250 ml of ethyl acetate and grind for 5 minutes in a mixer.

3.4 Add 50 g of Na_2SO_4 and grind for 5 minutes. Filter a minimum quantity of 50 ml through a fluted filter. The filtrate is "solution B".

Clean up

3.5 Pipette 50 ml of "solution B" into a 100-ml round-bottom flask. Evaporate to dryness, using vacuum. Adsorb the residue in 25 ml of acetonitrile and keep the flask, with swirling, for 10 seconds in the sensor bath.

3.6 Wash the solution with 50 ml of hexane in a separating funnel. Shake for two minutes. Discard the hexane layer.

3.7 Add 50 ml of hexane to the acetonitrile layer. Shake for one minute. Discard the hexane layer.

3.8 Draw off the acetonitrile layer in a round-bottom flask. Rinse the flask with 5 ml of acetonitrile. Evaporate the solution to dryness. Adsorb the residue in 5.0 ml of acetone. This is "solution I".

Column chromatography

3.9 Fill a column [illegible] cm. diam. [illegible] with silica gel of 0.05 - 0.2 mm particle size, [illegible] Close the bottom end of the column with [illegible] Wash the column with 10 ml of petroleum ether at a rate of 1 ml/min. Discard the [illegible]

3.10 Add 25.0 ml of petroleum ether (40 - 65°C) to "solution C". The solution then obtained is called "solution D".

3.11 Introduce "solution D" into the column, using 25.0 ml of petroleum ether.

3.12 Flush successively with: 2 x 25.0 ml of 20% acetone/petroleum ether
2 x 25.0 ml of 15% ethanol/petroleum ether.

3.13 Elute with: 2 x 30.0 ml of 30% ethanol/petroleum ether.

3.14 Evaporate the eluate to dryness. Add 5 ml of hexane and evaporate to dryness. Dissolve the residue in 5.00 ml of acetonitrile while swirling for 10 seconds in the sensor bath. This is "solution E".

Derivatisation

3.15 Pipette 1 ml of "solution E" into an amber-coloured bottle of 10 ml (with screw-thread). Add 50 ul of HFBA. Close the bottle with a screw-cap which contains a teflon-coated rubber disc. Allow the bottle to stand for ten minutes at room temperature.

3.16 Add 4 ml of water, 0.5 ml of saturated Na_2CO_3 solution, and 5.00 ml of hexane. Shake for 30 seconds! This is "solution F". NOTE: Transfer the hexane layer immediately to another bottle.
Treat the bottles one at the time.

Gas chromatography

3.17 Fill a 20-cm glass, silanised column (int.diam. 3 mm) with the column packing.

3.18 Disconnect the detector from the column. Stabilise the column by heating it for 24 hours at 180°C, while passing through 30 ml of nitrogen gas per minute.

3.19 Connect the detector. Set the temperature of the column at 160°C, that of the detector at 220°C and that of the injection port at 220°C. Set the rate of the carrier gas at 50 ml/min and that of the supplementary gas at 10 ml/min.

3.20 Inject and chromatograph 4 µl of the supernatant liquids of the CPU standard solutions (from 0.01 µg/ml of CH_3CN to 0.1 µg/ml of CH_3CN), which have been treated in accordance with 3.15 and 3.16.

3.21 Determine the peak area of the CPU derivative. Plot this against the corresponding injected quantities of µg of CPU per ml of final solution.

Draw a line through the points. This is the calibration curve.

3.22 Inject 4 µl of the supernatant liquid of "solution F" and chromatograph.

The peak area should fall inside the calibration curve. If it does not fall inside the calibration curve, then dilute the final solution further with hexane.

3.23 Determine the peak area of the CPU derivative.

4. CALCULATION

The quantity of CPU is:

$$\frac{a \times v}{v} \times f = \quad \text{mg/kg}$$

a = quantity of CPU read from the calibration curve, in µg per ml of final solution

v = dilution factor = 125

v = weighed quantity of sample, in g

f = dilution factor (of the final solution see 3.22)

HLA 6012-271

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26/APR/1989

RES 031

GAS CHROMATOGRAPHIC DETERMINATION OF 2,6-DIFLUOROBENZOIC ACID IN SOIL

1. PRINCIPLE

After extraction with diethylether 2,6-difluorobenzoic acid is derivatized with pentafluorobenzylbromide. After derivation a clean-up on a silicagel column is required. The pentafluorobenzyl derivate of 2,6-difluorobenzoic acid is separated from impurities by means of gas chromatography on a capillary column with DB-1 as stationary phase.
The derivate is detected with an electron capture detector.
The content of 2,6-difluorobenzoic acid is determined by comparing the peak height of the sample with that of a standard solution.

2. REQUISITES

Reagents

- diethylether, pure
- n-hexane, spectrograde
- acetone (Baker analyzed reagent)
- bidistilled water
- sodium sulfate (Baker grade, anhydrous)
- filter pulp (Schleicher und Schull 122)
- sulfuric acid 95-97% (Baker analyzed reagent)
- pentafluorobenzylbromide (Pierce)
- sodium carbonate (Baker analyzed reagent)
- 2,6-difluorobenzoic acid ARS
- tetradecane (Baker grade)
- toluene (Baker analyzed reagent)
- silicagel, disposable columns (Baker 7086-6)
- 10% H_2SO_4 (dissolve 100 ml H_2SO_4 in 1000 ml of bidist. water)
- 1% PFBBr (add to 1.0 ml of pentafluorobenzylbromide 100 ml of acetone

- toluene/hexane 1:6 (add to 100 ml of toluene 600 ml of hexane)
- toluene/hexane 9:1 (add to 900 ml of toluene 100 ml of hexane)
- helium, pure (carrier gas)
- nitrogen, pure (make-up gas)

Apparatus

- 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 um DB-1 and equipped with an uncoated, deactivated retention gap (length 0.5 m, i.d. 0.53 mm).
- rotavapor.
- syringe suitable for on-column injection on capillary columns, 3 µl.
- homogeniser (e.g. Townson and Mercer).
- ultrasonic bath.

3. PROCEDURE

Gas chromatography

3.1 Adjust the pressure of the carrier gas and the pressure of the make-up gas to 100 kPa.

Set the temperature of the column oven at 110°C, start the temperature program at injection, with a rise in temperature of 15°C/min. up to 160°C. Keep at 160°C for 5 min., program the temperature to increase by 30°C/min. up to 250°C. This is the final temperature; maintain it for 5 min.

Set the detector temperature at 280°C.

Set the secondary cooling at 10 sec. after injection.

Extraction

3.2 Add to a representative sample of 50 g, 50 ml 10% H_2SO_4, and place this for 5 minutes in an ultrasonic bath.

3.3 Macerate the sample with 200 ml diethylether in a homogeniser for 4 minutes.

3.4 Add 100 g Na_2SO_4 and 2 g of filter pulp. Macerate for 1 minute.

3.5 Filter about 50 ml of the diethylether layer through a fluted filter.

3.6 Pipette 50 ul tetradecane into a 100-ml round bottom flask and pipette 5,00 ml of the filtrate into the flask.

3.7 Evaporate to dryness under vacuum at 40°C.

Derivation

3.8 Dissolve the residue in 5 ml of 1% PFBBr solution, add a few crystals of Na_2CO_3 and derivate for 30 minutes in a water bath at 50°C.

3.9 Evaporate to dryness under vacuum at 40°C.

Clean-up

3.10 Wash a silicagel column with the aid of air pressure with 2 ml of toluene/hexane in a ratio of 1:6.

3.11 Dissolve the residue from 3.9 in 2 ml of toluene/hexane in a ratio of 1:6 and introduce on the silicagel column.

3.12 Wash the column with 6 ml of toluene/hexane in a ratio of 1:6.

3.13 Elute the pfb-derivate of 2,6-dfba from the column with 4 ml of toluene/hexane in a ratio of 9:1 into a 5-ml volumetric flask with the aid of air pressure. Make up to volume with toluene/hexane in a ratio of 9:1. This is "solution A".

Calibration line

3.14 Prepare six solutions containing respectively about 0, 0.025, 0.050, 0.075, 0.100 and 0.125.

3.15 Introduce with a pipette 1.00 ml of each solution in a 100-ml round bottom flask which contains 50 µl of tetradecane. Evaporate to dryness under vacuum at 40°C.

3.16 Carry out the derivation and clean-up as described in section 3.8 - 3.13.

3.17 Dissolve the residue in 5.00 ml of toluene/hexane in a ratio of 9:1, inject 1 µl of these solutions and chromatograph.

3.18 Determine the height of each 2,6-dfba derived peak.

3.19 Plot the peak heights found against the corresponding concentrations of 2,6-dfba in the injected solutions in µg/ml. Draw a straight line through the points. This is the calibration line.

Sample

3.20 Inject 1 µl of "solution A" and chromatograph.

3.21 Determine the height of the 2,6-dfba derived peak. Read off from the calibration line the concentration of 2,6 dfba which corresponds to this peak.

4. CALCULATION

The 2,6-difluorobenzoic acid content is:

$$\frac{p \times v}{\quad} \quad \mu g/kg$$

- p = concentration read off from the calibration line, in µg/ml
- v = extraction volume of the sample = 200 ml
- = weight of the sample, in kg (3.2).

5. REMARKS

5.1 The retention time of the 2,6-difluorobenzoic acid derived peak is approx. 7 min.

5.2 The amount of reagent (PFBBr) necessary may vary, depending on the type of soil, 5 ml of a 1% PFBBr solution should be sufficient in all cases.

5.3 The relative high amount of PFBBr reagent makes clean-up necessary for samples as well as calibration solutions.

5.4 The derivation can, in most cases, also be performed with 2 ml of a 0.2% PFBBr solution, in this case the clean-up step can be omitted. After section 3.9, the residue can be solved in 5.0 ml of the toluene/hexane (9:1) mixture.