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Method for Gas Chromatographic Determination of Residues of the Fungicide FOLICUR (HWG 1608) in Plant Materials, Soil and Water

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Summary

A method is described for determining residues of the fungicide FOLICUR (active ingredient (AI): HWG 1608) by gas chromatography (GC) in plant material, soil and water. The AI is extracted from plant materials with acetone or acetone/water (3:1) and from soil with methanol/water (7:3). After filtration, the extracts are shaken with dichloromethane. The AI is extracted directly with dichloromethane from water samples. The dichloromethane extracts from plant material are cleaned up via column chromatography, first on silica gel and then on polystyrene gel Bio Beads S-X 3. Column chromatography cleanup on silica gel is not necessary for the dichloromethane extracts from soil and water samples.

The AI is determined by GC using a thermionic N/P detector.

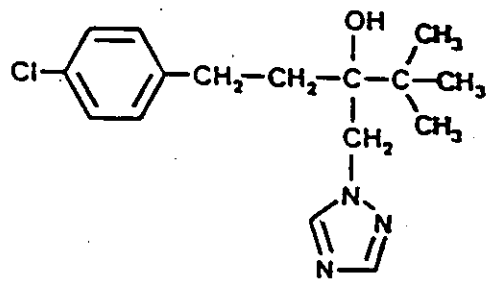
The recoveries were within a range of 80 - 104% for samples spiked with from 0.005 mg/L to 5 mg/kg. The lowest determined concentration of HWG 1608 was 0.05 mg/kg in plant material and soil samples and 0.005 mg/L in water samples.

1. Introduction

The fungicide FOLICUR contains the active ingredient (AI) HWG 1608, which has the following chemical and physical properties:

Chemical Designation: alpha-[2-(4-chlorophenyl)ethyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol

Structural Formula:



Empirical Formula: $C_{16}H_{22}ClN_3O$

Molecular Weight: 307.8

Appearance: colorless crystals

Melting Point: 104.7°C

Vapor Pressure: $<10^{-5}$ mbar at 20°C

Solubility at 20°C:	water	0.032
(g/1000 mL solvent)	dichloromethane	>200
	n-hexane	2 - 5
	2-propanol	100 - 200
	toluene	50 - 100

Hydrolysis Stability:	at pH 4 >1 year
(Half-Life at 20 °C)	at pH 7 >1 year
	at pH 9 >1 year

2. Description of Method

The AI is extracted from grain samples (green foliage, kernels, straw) and from peanut samples (shells, nuts) with acetone/water (3:1). Other experimental plant material is extracted exclusively with acetone. In the case of soil samples, a hot extraction with methanol/water (7:3) is performed. The extracts of plant samples are saturated with sodium chloride after filtration; then the AI's are extracted with dichloromethane. In the case of soil extracts, the methanol is evaporated after filtration and the aqueous residue is shaken with dichloromethane. Water samples are directly extracted with dichloromethane.

The dichloromethane extracts dried with sodium sulfate are evaporated to dryness. In the case of plant material, the residue is first cleaned up via column chromatography on silica gel and subsequently via gel chromatography on polystyrene gel Bio Beads S-X 3. In the case of soil and water samples, cleanup via column chromatography on silica gel is not necessary.

After concentrating the eluates containing the AI, the AI is determined via GC with a thermionic nitrogen-phosphor detector (TID).

3. Equipment

high rpm blender, e.g., Polytron, KINEMATICA, Kriens, Switzerland

glass flasks, 1000 mL, wide neck, with ground glass joint

vacuum filter flasks, 1000 mL

porcelain Buchner funnel, 110 mm ID with fast filter paper, 110 mm diameter

glass funnel, 100 mm diameter

round-bottomed flasks, 100 mL, 250 mL, 500 mL, 1000 mL with ground neck

reflux condenser

heating mantels, for 1000 mL round-bottomed flasks

graduated cylinders, 100 mL, 250 mL, 500 mL

volumetric flasks, 25 mL, 50 mL, 100 mL with ground neck

graduated pipettes, 10 mL, 25 mL

pipettes, 1 mL, 3mL, 5 mL, 10 mL

separatory funnels, 500 mL, 1000 mL with ground glass stopper

test tubes with ground glass joint, 10 mL

rotary vacuum evaporator with water bath, bath temperature 40°C

chromatography column: 17.5 mm ID, length 340 mm, elongated discharge with Teflon cock

gel chromatograph GPC Autoprep 1002 (Analytical Biochemistry Laboratories, Inc., Columbia, MO 65201; distributor: N. Foss Electric A/S GmbH, Waidmannstr. 12b, D-2000 Hamburg 19, FRG).

chromatography column: 25 mm ID, length 600 mm, packed with Bio Beads S-X 3, 200-400 mesh, pretreated to allow for swelling in elution mixture 2, packing height: 320 mm, elution rate: 5.0 mL/min

injection syringe with Luer-Lock connection, 10 mL

injection syringe, 10 µL

gas chromatograph with thermionic N/P detector (TID), e.g., Varian 3700 or HO 5890

integrator, e.g., Spectra Physics 4100 or LAS on HP 1000 A900

4. Reagents

acetone, for residue analysis

cyclohexane, for residue analysis

dichloromethane, for residue analysis

ethyl acetate, for residue analysis

n-hexane, for residue analysis

methanol, for residue analysis

toluene, for residue analysis

acetone-water mixture 3:1 (v/v)

elution mixture 1: n-hexane/ethyl acetate 2:8 (v/v)

elution mixture 2: cyclohexane/ethyl acetate 1:1 (v/v)

methanol-water mixture 7:3 (v/v)

AI standard solutions: 0.1 - 250 µg HWG 1608/mL ethyl acetate

sodium chloride, reagent grade

sodium sulfate, reagent grade, anhydrous

filter aid, e.g., Celite 545

silica gel 60: 0.063 - 0.200 mm, 70 - 230 mesh (MERCK No. 7734)

polystyrene gel: Bio Beads S-X 3, 0.037 - 0.074 mm, 200 - 400 mesh
(Bio-Rad Laboratories, No. 152 - 2750)

glass wool

cotton wool, chemically pure

helium 5.0

air, synthetic

nitrogen, 4:6

hydrogen, 5.0

5. Sampling

The analytical samples are obtained and prepared according to the instructions of sections VIII, IX and X of the DFG method collection on the residue analysis of pesticides (see Deutsche Forschungsgemeinschaft [German Research Assoc.], 1982).

6. Analytical Procedure

6.1 Extraction

6.1.1 Grain and Peanuts

To the sample material [50 g (G) green foliage of grain or 50 g (G) grain kernels or 25 g (G) grain straw or 50 g (G) shelled peanuts or 25 g peanut shells] in a 1 L glass flask, 400 mL acetone-water mixture (3:1) are added and homogenized for approx. 3 min with a blender. After adding approx. 15 g filter aid, the glass flask is swirled several times and the

suspension is filtered through a porcelain Buchner funnel under gentle vacuum. Container and filter solids are rinsed twice with 100 mL acetone-water mixture (3:1) each time. The filter solids are pulled dry and discarded. The filtrate is placed into a 1 L separatory funnel, saturated with approx. 40 g sodium chloride and then shaken with 100 mL dichloromethane. The lower aqueous phase is discarded after phase separation. The organic phase is drained into a 1 L round-bottomed flask and concentrated on a rotary evaporator to a volume of approx. 40 mL. After addition of 25 mL dichloromethane, the solution is dried with approx. 30 g sodium sulfate. It is then filtered through a funnel containing a cotton wool plug and an approx. 3 cm deep sodium sulfate layer into a 500 mL round-bottomed flask. The 1 L round-bottomed flask and the filter are rinsed 3 times with 50 mL dichloromethane each. The filtrate is evaporated to dryness on the rotary evaporator. The residue is further treated according to 6.2.

6.1.2 Plant Material with High Water Content

To the sample material [100 g (G) bananas or 50 g (G) banana peel or 100 g (G) grapes] in a 1 L glass flask, 300 mL acetone are added and homogenized for approx. 3 min in a blender. Further processing of the sample is performed as described under 6.1.1.

6.1.3 Must and Wine

100 g (G) (must, wine) are placed into a 1 L separatory funnel. After addition of 200 mL acetone, the solution is saturated with approx. 40 g sodium chloride and then shaken with 100 mL dichloromethane. After phase

separation, the lower aqueous phase is discarded. The organic phase is processed as described under 6.1.1.

Note: In the case of must, the sample is first vacuum filtered through a porcelain Buchner funnel with the addition of approx. 15 g filter aid to prevent emulsions.

6.1.4 Soil

In a 1 L round-bottomed flask containing 300 mL methanol-water mixture (7:3), 50 g (G) soil are heated for 4 hours under reflux. After cooling, the suspension is filtered under gentle vacuum through a porcelain Buchner funnel containing approx. 15 g filter aid. Flask and filter solids are rinsed twice with 50 mL methanol-water mixture (7:3) each time. The filter solids are pulled dry and discarded. The filtrate is concentrated on the rotary evaporator to an aqueous residue (approx. 100 mL); this residue is placed into a 250 mL separatory funnel. After rinsing the flask with dichloromethane, the aqueous residue is shaken three times with dichloromethane (100, 50, 50 mL). The organic phases are filtered individually over sodium sulfate (glass funnel with cotton wool plug and a 3 cm deep sodium sulfate layer) into a 500 mL round-bottomed flask. After rinsing the sodium sulfate three times with 25 mL dichloromethane, the filtrate is evaporated to dryness on the rotary evaporator. The residue is further processed according to 6.3.

6.1.5 Water

200 mL (G) water [e.g., leachate according to BBA pamphlet No. 37 (Biologische Bundesanstalt), 1980)] are shaken three times with dichloromethane (100, 100, 50 mL) in a 500 mL separatory funnel. In case other water volumes are used, the samples must be extracted with corresponding dichloromethane volumes. The organic phases are filtered individually over sodium sulfate (glass funnel with cotton wool plug and an approx. 3 cm deep sodium sulfate layer) into a 500 mL round-bottomed flask. The sodium sulfate is rinsed with approx. 25 mL dichloromethane. The filtrate is evaporated to dryness on the rotary evaporator and the residue is further processed according to 6.3.

6.2 Column Chromatography on Silica Gel

The chromatography column is packed in the following sequence: 10 mL toluene, cotton wool plug, 20 g silica gel slurried in toluene (filling height approx. 170 mm), approx. 10 mm sodium sulfate layer. The toluene is drained until it reaches the sodium sulfate layer.

The residue from either 6.1.1, 6.1.2 or 6.1.3 is dissolved in 10 mL toluene and the solution is placed on the column with a pipette. After the solution has trickled down to the sodium sulfate layer, the flask is rinsed with 20 mL ethyl acetate. Then 5 mL hexane are added, the flask is swirled once more, and this solution (25 mL) is placed on the column. This process is repeated once. The column is rinsed with the rinsing solutions (10, 25, 25 mL). Then the AI is eluted with 150 mL elution mixture 1. The eluate

is collected in a 250 mL round-bottomed flask and evaporated to dryness on the rotary evaporator. The residue is further processed according to 6.3.

6.3 Gel Chromatography (GPC)

The residue from either 6.1.4, 6.1.5 or 6.2 is dissolved in 10 mL elution mixture 2. 7 - 8 mL of the solution are injected with a 10 mL injection syringe into the 5 mL sample loop of the gel chromatograph. The gel chromatograph is set to the elution conditions previously determined with a standard solution of HWG 1608 (approx. 40 ug/mL).

Using polystyrene gel Bio Beads S-X 3, an elution range of 115 - 160 mL was determined for HWG 1608 using elution mixture 2 at an elution rate of 5.0 mL/min. After approx. 500 samples have been run, the elution range should be checked. When using a new gel column, the elution range must be determined again.

The AI eluates (elution range: 115 - 160 mL) are collected in a 100 mL round-bottomed flask and evaporated to dryness on the rotary evaporator.

6.4 Gas Chromatographic Determination

The residue from 6.3 is dissolved in a specified volume (V_{End}, e.g. 5 mL) of ethyl acetate and the solution is transferred into a test tube with ground glass joint. An aliquot of this solution (V_i) is injected into the gas chromatograph. If the AI content is too high, an aliquot of V_{End} is diluted with ethyl acetate according to the sensitivity of the detector so

that the peak areas of analytical sample (F_A) and standard (F_{St}) are of comparable size. Each injection is repeated for control purposes.

Measuring Procedure and Conditions

Instrument	Varian 3700	Varian 3700	HP 5890	Carlo Erba HRGC 5300	
Column	Glass 135 cm, 3 mm I.D.	Glass 180 cm, 3 mm I.D.	Glas, 150 cm, 2 mm I.D.	Fused Silica *Capillary*	
				HP-5 10 m, 0,530 mm I.D.	
Column Packing	3 % SP 2100 *on* Supelcoport 80/100 mesh			2,65 µm *Film Thickness*	
Temperature Injector	280 °C	280 °C	300 °C	300 °C	
Column	200 °C	235 °C	235 °C	60 °C 1 min isothermic	
Program				60 °C → 30 °C/min → 180 °C	
				180 °C 3 min isothermic	
				180 °C → 15 °C/min → 240 °C	
				240 °C 8 min isothermic	
Detector	300 °C	300 °C	300 °C	300 °C	
Carrier Gas	N_2 ca. 30 ml/min	N_2 ca. 45 ml/min	N_2 ca. 10 ml/min	He 6,6 ml/min 13 kPa	
Make-up Gas				He 25 ml/min 105 kPa	
Combustion Gases	H_2 4,5 ml/min	H_2 4,5 ml/min	H_2 2 ml/min	H_2 35 ml/min 13 kPa	
	Air 175 ml/min	*Air* 175 ml/min	*Air* 100 ml/min	*Air* 350 ml/min 93 kPa	
Attenuation	1×10^{-11}	1×10^{-11}	1	1	
Injection Volume	5 µl	5 µl	3 µl	1 µl	
Retention Time	~ 4 min	~ 3,2 min	~ 2,9 min	~ 10,6 min	

7. Evaluation

7.1 Evaluation Procedure

Quantitative evaluation is performed via integrator or LAS by determination and comparison of the peak areas of the analytical solutions with the peak areas of standard solutions (external standard method). Equal volumes of the analytical and the standard solutions were injected. The linearity range tested for HWG 1608 was from 0.2 - 30 ng (Fig. 1).

7.2 Recoveries and Lowest Determined Concentration

The recoveries were determined in recovery tests, in which specified amounts of HWG 1608 dissolved in 1 - 2 mL ethyl acetate were added to untreated control samples prior to extraction. The results of the recovery determinations are summarized in Table 1. For the recovery determination in soil, 3 different soils were used:

Soil Designation	C Content (%)	SM (%)*	pH
standard soil 2.1**	0.81	10.0	5.7
standard soil 2.2**	2.71	12.9	5.8
standard soil 2.3**	0.98	24.8	7.0

* SM = suspended material < 0.02 mm

** according to BBA pamphlet No. 37

The lowest determined concentration for HWG 1608 in plant material and soil samples was 0.05 mg/kg and in water samples 0.005 mg/L.

7.3 Calculation of Residue Amounts

The amount of residue R, expressed in mg HWG 1608/kg sample material is calculated as follows:

$$R = \frac{F_A \times V_{End} \times W_{St} \times V_{R1} \times F}{F_{St} \times V_{R2} \times V_i \times G}$$

Where

G = weight of the analytical sample (g, mL for water)

V_{End} = final volume of the analytical solution (e.g., 5 mL)

V_{R1} = volume of the solution for gel chromatography (e.g., 10 mL)

V_{R2} = aliquot of V_{R1} injected into the gel chromatograph (e.g., 5 mL)

V_i = aliquot of V_{End} injected into the gas chromatograph (e.g., 5 µL)

F_A = peak area obtained from V_i for the analytical solution (µV/s)

F_{St} = peak area obtained from W_{St} for the standard solution (µV/s)

W_{St} = amount of AI injected with the standard solution (ng)

F = recovery factor determined by the analyst = $\frac{100}{\text{recovery}}$

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8. Literature

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[signed]

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