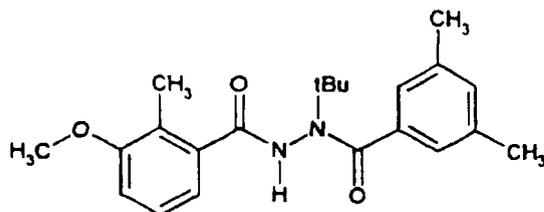


Bayer AG  
PF-E/MR

## 2 Introduction

The active ingredient RH 2485 is used as insecticide and has the following chemical structure:



RH 2485

**Chemical Name:** N'-t-butyl-N'-(3,5-dimethylbenzoyl)-  
N-(2-methyl-3-methoxybenzoyl)hydrazine

**Total Formula:** C<sub>22</sub>H<sub>26</sub>N<sub>2</sub>O<sub>3</sub>

**Molar Mass:** 368.5 g/mole

**Appearance:** white powder

**Solubility [g/L at 20°C]:**

Water	0.00193
Acetone	100.2
Dichloromethane	45.4
Methanol	152.7
2-Propanol	39.4
Xylene	2.9

**Melting Point [°C]:** 203.8 - 208.4

**Partition Coefficient, log Pow:** 3.72  
(n-Octanol/Water, 25°C)

**Vapour Pressure:** < 1.33 · 10<sup>-7</sup>  
[hPa at 25°C] 2.48 · 10<sup>-8</sup>

**Hydrolytic Stability:** pH 4 > 500  
**Half-Life Period at 40°C [h]** pH 7 > 500  
pH 9 > 500

Bayer AG  
PF-E/MR

### 3 Multi-Residue Methods

According to the Guideline [3], multi-residue methods [4, 5, 6] have to be tested for their suitability as analytical method. Because of its low vapour pressure and its chemical structure, RH 2485 is not suitable for GC-analysis, so that the two methods [4] and [5] could not be used for determination of RH 2485.

The determination of RH 2485 according to method [6] gave unsatisfactory results. Due to low sensitivity at 240 nm (figure 18), reliable quantification of RH 2485 was impossible for soil 2.2 and soil Höfchen. Additionally, matrix interferences with the same retention time as RH 2485 disturbed quantification at 220 nm, so that the recovery requests could not be fulfilled. This is demonstrated for sample fortification with 98 µg/kg of RH 2485 (figures 19 and 20).

### 4 Test System

The method was validated using soil 2.2 of LUFA Speyer and soil from trial station Höfchen, plot 4011. The soil samples were classified according to DIN and USDA specifications. The textural classification of the soils is summarized in Table 1.

The data are reported in the appendix (Table 6 to Table 7).

Table 1 Soil Parameters

Soil	Type of Soil	Origin of Classification
LUFA 2.2	weak loamy sand	DIN
Höfchen	heavy loamy silt	DIN

### 5 Instruments

Extraction Equipment: Soxtec HT 6  
Tecator  
S-26321 Höganäs, Sweden

Extraction Tubes: 26 x 60 mm  
Ref. No. 350 220  
Schleicher & Schüll  
D-37586 Dassel

Evaporator: Turbo Vap LV  
custom made for 50 mL centrifuge tubes  
Zymark GmbH  
D-65510 Idstein

Balances: PC 4400 and PM 4800  
Mettler Instruments GmbH  
D-35387 Giessen

Ultrasonic Bath: Transsonic 890/H  
Heinrich Faust  
D-51145 Cologne

Evaporation Tubes: 50 mL centrifuge tubes  
Zymark GmbH  
D-65510 Idstein

Bayer AG  
PF-E/MR

Vac Elut: TYP SPS 24  
Varian GmbH  
D-64289 Darmstadt

Envi-Carb SPE tubes: 0.5 g, 6 mL  
Order No.: 57094  
Supelco  
Bellafonte, PA, 16823-0048 USA

Rotavapor: Rotavapor RE  
Büchi GmbH  
D-73001 Göppingen

Liquid Chromatograph: HP 1090  
Hewlett-Packard  
D-40880 Ratingen

UV-Detector: UV 2000  
Thermo Separation Products  
D-64291 Darmstadt

DAD  
Hewlett-Packard  
D-40880 Ratingen

HPLC columns : Superspher 100 RP 18, 250 x 4 mm  
Order No.: 18058  
E. Merck  
D-64271 Darmstadt

Zorbax SB-CN, 250 x 4.6 mm  
Order No.: 880975905  
Bischoff Analysentechnik GmbH  
D-71229 Leonberg

Volumetric flasks, pipettes and other instruments commonly used in the laboratory.

## 6 Reagents

Acetonitrile: Riedel de Haen  
Order No. 34998  
D-30826 Seelze

Deionized Water: purified in a Milli-Q unit

Ethanol: E. Merck  
Order No.: 1.00983.6010  
D-64271 Darmstadt

Methanol: Promochem  
Order No.: 3041  
D-46485 Wesel

Phosphoric Acid: E. Merck  
Order No.: 1.00552.0250  
D-64271 Darmstadt

Bayer AG  
PF-E/MR

Hydrochloric Acid:        Fixanal, 1 m HCL  
                                  Order No.: 38282  
                                  Riedel de Haen  
                                  D-30926 Seelze

## 7 Reference Substance

Name of the Compound:    RH 2485  
Certificate of Analysis:    960507ELB01  
Purity:                      99.4% (HPLC, NMR, MS and GLC-headspace for determination of  
                                      volatile impurities)  
Expiry date:                March 2000

The stock solutions of the reference substance are prepared in acetonitrile. From this the measuring solutions are prepared by dilution with water/acetonitrile (50/50; v/v).

The concentration range of the measuring solutions was between approx. 0.1 and approx. 2.5 µg/mL.

## 8 Safety Precautions

While processing this method the German guidelines for laboratories of the professional association (e.g. leaflet M006) or similar guidelines in other countries have to be considered.

The following, according to the German *Gefahrstoffverordnung* as very toxic, toxic or less toxic rated plant protection compounds and solvents are used. This classification is based on German guidelines and has to be adjusted to the national guidelines of other countries while using the method outside of Germany.

treated as very toxic: RH 2485 (not fully investigated)

toxic:                        Acetonitrile, Methanol

less toxic:                Hydrochloric Acid, Phosphoric Acid, Ethanol

While working with these substances the relevant safety regulations have to be considered (see R- and S-rules).

Bayer AG  
PF-E/MR

## **9 Performance of Analysis**

### **9.1 Fortification**

The method is validated by analyzing control samples and control samples fortified prior to the extraction procedure at and above the limit of quantification.

Sample fortification should be performed in such a way that no less than 100 µL and no more than 1 mL of the standard solution is used. The preparation of the fortification standards is described in section 7.

The delay between fortification and the beginning of the extraction is 1 hour, to allow the standard to be sucked into the soil.

### **9.2 Extraction**

Soil samples of 25 g are extracted in a Soxtec extraction device with boiling methanol/water/1 m HCl (900/100/5, v/v). The oil-bath temperature is set to 200 °C.

Soil samples are weighed into an extraction thimble and covered with a defatted cotton wool plug. 40 mL of the extraction solvent and some boiling chips are placed into aluminium cups. Thimbles and cups are inserted in the Soxtec extraction device. The extraction time takes 1 hour. Afterwards the thimbles are placed in rinse position for 30 minutes until the extraction is terminated.

The residue is flushed quantitatively into a 50 mL centrifuge tube by rinsing the aluminium cups 2 times with about 5 mL of ethanol. The extract is evaporated to dryness in a Turbo-Vap evaporator at 50°C and reconstituted in 5 mL of methanol/water (40/60; v/v).

### **9.3 Carbon Solid Phase Extraction**

A SPE cartridge, packed with 0.5 g of Envi-carbon is preconditioned with 10 mL of methanol and 10 mL of water using a Varian SPS vacuum manifold or similar apparatus. The extract from 9.2 is added to the SPE cartridge and the flask is rinsed with another 5 mL of methanol/water (40/60; v/v). This solution is added to the cartridge as well. After that the cartridge is washed with additional 10 mL of methanol/water (40/60; v/v). All washing solutions are discarded. RH 2485 is eluted with 20 mL of methanol. The methanol solution is evaporated to dryness and reconstituted in 2 mL of acetonitrile/water (50/50; v/v). The sample is now ready for HPLC quantification.

### **9.4 Storage Stability of the Extracts**

The extracts of the samples are stable for the time of analysis. If an interruption of the analysis is necessary, extracts have to be stored in solution in a refrigerator.

Bayer AG  
 PF-E/MR

### 9.5 High Performance Liquid Chromatographic Measurement

An aliquot of the extract of 9.3 is injected into the high performance liquid chromatograph.

High performance liquid chromatographic instrumentation and conditions are as follows:

Columns\*: a: Superspher 100 RP-18, 250 x 4 mm

b: Zorbax SB-CN, 250 x 4.6 mm

Oven temperature: 40°C

Mobile phase: A: Water + 1.0 mL phosphoric-acid per litre  
 B: Acetonitrile

\* Two different columns and gradient systems are used. With these two columns of different selectivity it is possible to avoid the detection of impurities as residues of RH 2485.

**Table 2** Gradient for column a: Superspher 100 RP-18, 250 x 4 mm

Time [min]	0	7	10	15	18	20
% A	35	35	10	10	35	35
% B	65	65	90	90	65	65

Runtime: 20 min Retention time: RH 2485: 5.6 min.

Flow: 1.0 mL/min Detector wavelength: 220 nm

Injection volume: 50 µL

**Table 3** Gradient for column b: Zorbax SB-CN, 250 x 4.6 mm

Time [min]	0	10	20	23	26	29	34
% A	80	60	60	10	10	80	80
% B	20	40	40	90	90	20	20

Runtime: 34 min Retention time: RH 2485: 22.8 min.

Flow: 1.0 mL/min Detector wavelength: 220 nm

Injection volume: 50 µL

Bayer AG  
PF-E/MR

## 10 Calculation of Residues

For quantification of the residue content in soil a comparison to external standard solutions of RH 2485 in water/acetonitrile (50/50; v/v) is made.

The residues of RH 2485 in wet soil expressed in  $\mu\text{g}/\text{kg}$  were calculated by applying the following equation:

$$C_{\text{ai}} = \frac{A_{\text{p}} \cdot C_{\text{s}}}{A_{\text{s}}} \cdot \frac{V_{\text{end}}}{M}$$

The recovery [%] for wet soil is calculated to the following equation:

$$R = \frac{C_{\text{ai}} \cdot 100}{C_{\text{Soil Spiked}}}$$

$A_{\text{p}}$	=	peak area of the sample solution [area counts]
$A_{\text{s}}$	=	peak area of the standard solution [area counts]
$C_{\text{s}}$	=	concentration of the standard solution [ $\mu\text{g}/\text{L}$ ]
$M$	=	mass of the soil sample [kg]
$C_{\text{ai}}$	=	concentration of active ingredient in soil [ $\mu\text{g}/\text{kg}$ ]
$V_{\text{end}}$	=	final volume of the sample solution [L]
$R$	=	Recovery [%]
$C_{\text{Soil Spiked}}$	=	concentration of the test substance spiked [ $\mu\text{g}/\text{kg}$ ]

Bayer AG  
PF-E/MR

### Appendix

Figure 1 Flow Diagram of Analysis Procedure

Extract 25 g of soil using a Soxtec hot extraction equipment with 40 mL of boiling methanol/water/1 M HCl (900/100/5; v/v) for 60 min and rinse for 30 min



Transfer the extract quantitatively into a 50 mL centrifuge tube



Evaporate to dryness in a Turbo Vap evaporator



Reconstitute in methanol/water (40/60; v/v)



Carbon solid phase extraction



Evaporate to dryness in a rotavapor



Reconstitute in acetonitrile/water (50/50; v/v)



HPLC