SUMMARY

A method has been developed for the determination of RH-117281 in drinking water and was validated in the concentration range from 0.05 µg/l to 1 µg/l. The analytical method employs a simple approach, involves minimum cost and time and requires commonly available equipment as requested by the guideline for monitoring purposes.

RH-117281 was extracted from 500 ml drinking water by partition with ethyl acetate. The organic phase was concentrated in a rotary evaporator and the residue was dissolved in 2 ml ethyl acetate. Separation and quantification was performed by gas chromatography (GC) using an electron capture detector (ECD).

The analytical method was validated by fortifying drinking water samples at 0.05, 0.1, 0.5 and 1.0 µg/l. Samples were run in triplicate. RCC Ltd drinking water was used for the fortification and as control sample.

Limit of detection (LDC): 0.01 µg/l
Limit of determination (LDM): 0.05 µg/l
Confirmatory method is GC/MS with detection on masses 187 and 258.

1 OBJECTIVE

The objective of this study is to develop an analytical method for determination of RH-1117281 residues in drinking water.

2 MATERIALS AND METHODS

2.1 TEST SUBSTANCE

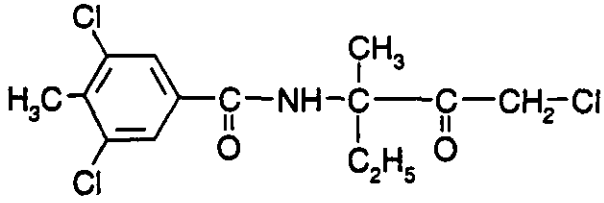
The data are listed as given by the sponsor.

Experimental Fungicide Name: RH-117281

Chemical Name (IUPAC): 3,5-dichloro-N-(3-chloro-1-ethyl-1-methyl-2-oxopropyl)-4-methylbenzamide

Generic Name: Zoxamide (proposed)

Structural Formula:



Batch: Lot Number: ELM 1157
Synthesised by Rohm and Haas Company Research Laboratories, Spring House, PA 19477/ U.S.A.

Expiration Date: 11-NOV-2001

Purity: 97.9%

Solubility at 20 °C: 0.67 ppm in water

Vapor Pressure:	$< 1 \times 10^{-7}$ torr
Appearance:	White powder
Octanol/Water Partition:	logP = 3.76
Soil Partition Constant:	Average K_{OC} = 1224
DT-50 in Soil:	7 to 14 days
Hydrostability:	Half-life at 25 °C and pH 4 = 14.88 days Half-life at 25 °C and pH 7 = 16.45 days Half-life at 25 °C and pH 9 = 5.52 days
Photostability:	Aqueous half-life = 7.8 days Soil half-life = ~ 10 days
Degradation in Soils:	Degrades rapidly
Storage:	Store frozen
Safety Precautions:	According to routine hygienic procedures, as far as appropriate under the supervision of an authorized safety expert.

2.2 TEST SYSTEM

The water used for the spiking and recovery experiments was drinking water as available at RCC Ltd in CH- 4452 Itingen/Switzerland.

To characterize the drinking water the following parameters were determined:

Total Hardness

The total water hardness was determined by complexometric titration with Aquameter®.

The beaker was first rinsed for several times with drinking water and then filled to a volume of 5 ml. The titrant was added dropwise until the solution turned from red to blue, marking the end point of the titration. The drops were counted. Determination of water hardness was performed in triplicate.

One drop corresponds to 1°dH (German degree of hardness) or 0.18 mmol/l alkaline-earth ions. Hence, the total water hardness was calculated to be 11°dH or **1.98 mmol/l**.

Total Mineral Content

The total mineral content was determined by a gravimetric method.

Two aliquots of 500 ml drinking water were lyophilized in 1000 ml round bottom flasks. The dried residue was weighed to be **0.58 g/l**.

pH-Value

The pH-value of the drinking water, measured by means of a calibrated pH-meter with glass electrode, was **pH 7.35**.

2.3 REAGENTS AND APPARATUS

Reagents and Solvents

All reagents and solvents were of pesticide residue analytical grade, if not specified otherwise.

Acetonitrile (HPLC-grade):	Baker no. 9017
Ethyl acetate:	Baker no. 9260-03
Buffer pH 7:	Baker no. 5656 (for calibration of pH-meter)
Buffer pH 10:	Baker no. 5655 (for calibration of pH-meter)
Aquamerck 1111 04 0001:	Merck, Testkit for hardness

Equipment

Rotary-evaporator:	Büchi 461 Water bath
Ultrasonic bath:	Bandelin Sonovex RK 102
Analytical balance:	Mettler HL 52, Mettler PM 400
pH-meter:	pH 96; WTW, Microprocessor
Glass electrode:	Schott N 6280
Volumetric flask:	Various sizes
Measuring cylinder:	Various sizes
Round-bottom flask:	250 ml, 1000 ml
Separatory funnel:	1000 ml
Funnel:	Various sizes
Glasvials:	Various sizes
Hamilton syringe:	Various sizes
Lyophylisator:	Freeze dryer Modulyo; Fa. Edwards

2.4 SOLUTIONS FOR FORTIFICATION AND CALIBRATION

Stock Solution 1

An aliquot of 17.38 mg (97.9%, see Section 2.1) of RH-117281 was placed in a volumetric flask and dissolved in 20 ml of ethyl acetate. This concentration corresponded to 0.8508 mg (100%) of RH-117281/ml ethyl acetate.

Stock Solution 2 99.96 µg/ml

2350 µl of the stock solution 1 was transferred to a 20-ml volumetric flask and filled to the mark with ethyl acetate.

Stock Solution 3 0.9996 µg/ml

100 µl of the stock solution 2 was transferred to a 10-ml volumetric flask and filled to the mark with ethyl acetate.

Example of Calibration and Fortification Solutions

Aliquots of stock solution 3 (St 3) were transferred to 2-ml vials and 1 ml of matrix-ethyl acetate* solution.

A) 9 µl of solution St 3 add 1.0 ml = 0.0089 µg RH-117281/ml
B) 13 µl of solution St 3 add 1.0 ml = 0.0128 µg RH-117281/ml
C) 25 µl of solution St 3 add 1.0 ml = 0.0244 µg RH-117281/ml
D) 140 µl of solution St 3 add 1.0 ml = 0.1228 µg RH-117281/ml
E) 330 µl of solution St 3 add 1.0 ml = 0.2480 µg RH-117281/ml
F) 430 µl of solution St 3 add 1.0 ml = 0.3006 µg RH-117281/ml

* The matrix-ethyl acetate solution was obtained by extracting 100 ml of tap water twice with 100 ml ethyl acetate each. The ethyl acetate extract was evaporated to dryness and the residue redissolved in 10 ml ethyl acetate. This solution served for the preparation of the calibration solution.

Fortification Solution = same as stock solution 3:

G) 100 µl of stock solution 2 (St 2) to 10 ml with ethyl acetate=0.9996 µg/ml

2.5 STABILITY OF SOLUTIONS

Standard and fortification solutions were newly prepared each time they were needed. Therefore no stability check was needed.

2.6 SAMPLES

2.6.1 Set of Samples

For the validation of the analytical method (see section 2.7) untreated water samples were amended in triplicate by addition of standard aliquots of RH-117281 at the following levels:

1.0 µg RH-117281/l

500 ml water aliquots (triplicate) were fortified with 500 µl of standard solution G.

0.5 µg RH-117281/l

500 ml water aliquots (triplicate) were fortified with 250 µl of standard solution G.

0.1 µg RH-117281/l

500 ml water aliquots (triplicate) were fortified with 50 µl of standard solution G.

0.05 µg RH-117281/l

500 ml water aliquots (triplicate) were fortified with 25 µl of standard solution G.

For each series one control sample (not amended) was worked up similarly as the amended samples.

2.7 ANALYTICAL METHOD

Extraction Procedure

A volume of 500 ml drinking water (V_S) was first transferred to a 1-l separatory funnel. Afterwards, the solution was extracted twice with 100 ml ethyl acetate by partitioning for about 2 min.

Thereafter, the combined organic phase was totally evaporated in a rotavap at about 30 °C.

For HPLC analysis the residue was dissolved in 2.0 ml (V_R) of ethyl acetate by means of an ultrasonic bath. Thereafter, the solution was transferred to a 2-ml volumetric flask and adjusted to 2.0 ml with ethyl acetate and analyzed by gas-chromatography (GC).

Conditions for GC-Analysis

The concentrations of RH-117281 in the extracts were quantified by Gas Chromatography (GC) under the following conditions:

Instrument:	HP 5890 Series II incl. HP AS 7673/A and EZ Chrom Chromatography Data System, Vers. 6.6		
Column:	DB1, 25 m x 0.25 mm, 0.25 µm film		
Pre-column:	Deactivated pre-column, 1.5 m x 0.53 mm		
Injection mode:	On column		
Carrier gas:	Helium		
Flow:	1.1 ml/min		
Oven temperature:	initial:	90 °C	1 minute
	rate 1:	10 °C/min	to 150 °C
	rate 2:	3 °C/min	to 250 °C
	rate 3:	30 °C/min	to 300 °C
	final:	300 °C	5 minutes
Injection volume:	1 µl (autosampler)		
Detection:	ECD		
Detector temperature:	300 °C		

The retention time of RH-117281 was about 23 minutes. Representative GC chromatograms are shown in Figures 1 to 8.

Conditions for GC-MS Analysis

GC-MS was used as confirmatory method.

Instrument:	HP 5890 Series II incl. HP AS 7673A
Column:	Rtx-1, 30 m x 0.25 mm, 0.25 µm film
Injection mode:	On column
Carrier gas:	Helium
Flow:	0.4 bar
Oven temperature:	Initial: 90 °C 1 minute Rate: 20 °C/min to 280 °C Final: 280 °C, 10 minutes
Ionization mode:	EI
Detection mode:	Positive ion detection
Scan mode:	SIM: m/z: 187, 258
Electron energy:	70 eV
Electron multiplier:	1.8 kV
Ionsource temperature:	183 °C
Transfer line temperature:	300 °C

2.8 EVALUATION OF RESULTS

Injected samples were quantified by peak area with reference to the calibration curve. The latter was obtained by correlation of the peak areas of the analytical standards with their corresponding concentrations of RH-117281 injected (µg/ml). Different regression models were tested (linear, power exponential and logarithmic regression). The best fitting model, the power exponential regression, was selected for the evaluation (see equation 1). The calibration curves of RH-117281 are shown on pages 21 and 22.

$$Y = b \cdot x^a \qquad (1)$$

where: X = Concentration of RH-117281 in injected sample (µg/ml)

Y = Detector response (peak area)

a = exponent

b = Constant

Therefore, the concentration (µg/ml) of RH-117281 in the injected sample was calculated by:

$$X = 10^{\left(\frac{1}{a} \cdot \log\left(\frac{y}{b}\right)\right)} \qquad (2)$$

The residue concentration (µg/l) of RH-117281 was calculated using the following equation:

$$C = \frac{X \cdot V_R}{V_S} \qquad (3)$$

where C = Concentration of RH-117281 in drinking water samples (µg/l)

X = Concentration (µg/ml) of RH-117281 in the injected sample derived from equation (2)

V_R = Reconstitution volume before injection into GC (2.0 ml)

V_S = Volume of drinking water Sample (0.5 l)

The recovery rate was calculated using the following equation (4):

$$R = \frac{C}{F} \cdot 100\% \qquad (4)$$

where: R = Recovery in percent
C = Total concentration found in µg/l
F = Fortification level in µg/l

Limit of Determination (LDM)

The limit of determination was 0.05 µg/l and was derived from the lowest fortification level (0.05 µg/l) at which acceptable recoveries in the range of 70-110% with a relative standard deviation not exceeding 20% were obtained.

However, the GC sensibility may be easily increased (at least twice) by injecting high volumes.

Repeatability

The precision under repeatability conditions, i.e. conditions where independent test results were obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time (September 4, 1998, September 8, 1998; cf. Table 1).

Calculations were performed with a commercially available computer program using up to fifteen decimal points. The results given in the tables are rounded. Thus, hand calculations may differ slightly from those presented due to rounding.