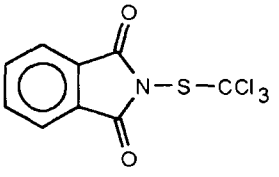
# 4 Materials and methods

## 4.1 Reference substance

**Folpet**



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| formula: | $C_9 H_4 O_2 N Cl_3 S$ |
| molar mass: | 296.6 |
| purity: | 99.3 % |
| supplier: | Makhteshim Chemical Works Ltd. |
| batch number: | 1471-16/3 |
| expiry date: | 05.01.95 |

The reference substance and stock solutions in toluene or acetonitrile were prepared on the day of their use and stored in a refrigerator at 0 – 5 °C.

## 4.2 Sample material

Tap water was used for all determinations after fortification with different amounts of folpet.

## 4.3 Method summary

The procedure used for determination of folpet is based on a method provided by the sponsor [1].

Water samples are extracted with dichloromethane. After evaporation of the solvent, folpet is determined by HPLC with UV (photodiodearray) detection.

## 4.4 Procedure for folpet determination

### 4.4.1 Apparatus

Round-bottom flasks (250 ml)
Separatory funnels (500 ml)
Volumetric flasks (20 ml)
Rotary vacuum evaporator with water bath (Büchi Rotavapor-R)
Microsyringes, 10 µl to 1000 µl
HPLC (Waters 600E with Waters Autosampler 717) equipped with photo-diodearray detector (Waters 996)
Glassware (separatory funnels and flasks) was cleaned with approx. 5 % hydrochloric acid, pure water, acetone and dichloromethane before use.

### 4.4.2 Reagents

Acetonitrile, HPLC grade (Baker No. 9017-54)
Dichloromethane for residue analysis (Promochem No. 3023)
Phosporic acid, p.a. 85 % (Merck No. 573)
Toluene for chromatography (Merck No. 8327)
Water (ultrapure) for HPLC
Hydrochloric acid (Riedel-de-Haën No. 30721)
Folpet stock solutions (0.5 or 1.0 mg/ml) were prepared by dissolving 5 to 25 mg folpet in 10 to 25 ml acetone or acetonitrile. Dilutions (0.1 to 10 µg/ml) for fortification experiments were prepared with ethanol or HPLC solvent (see 4.4.5). Dilutions for gaschromatographic standards were prepared from the acetone stocks solution with toluene.

### 4.4.3 Sample preparation

The same batch of tap water (3 – 4 litres) was used for all fortification experiments and was stored in a refrigerator before use.

### 4.4.4 Extraction

200 ml of the water sample was extracted three times with each 25 ml dichloromethane by shaking for approx. 1 min in a separatory funnel. The dichloromethane phase was collected in a 100 ml or 250 ml flask and the solvent was removed on a vacuum rotary evaporator (< 50 °C water bath temperature).

The residue was dissolved in 1 ml toluene (for gaschromatographic analysis) or 1 ml HPLC solvent (see 4.4.5). Since the toluene extract prepared for the first recovery experiment was used for HPLC analysis

instead, the solvent was removed completely under a stream of nitrogen and the residue was dissolved in 1 ml HPLC solvent.

4.4.5 Analysis of folpet by HPLC

Folpet was analysed by HPLC with UV detection under the following conditions:

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| *HPLC system* | Waters model 600E with Waters 717 autosampler |
| *Column* | Shandon Hypersil C18, 250 mm x 4 mm i.d. |
| *Temperature* | 40 °C |
| *Eluent* | acetonitrile/water (60:40, v/v) with 5 mM $KH_2PO_4$, adjusted to pH 4.5 (20 °C) with $H_3PO_4$ |
| *Flow* | 1 ml/min |
| *Injection volume* | 50 µl |
| *Detector* | Waters model 996 photodiodearray detector<br>For routine analysis, spectra are recorded from 200 nm to 250 nm. Processing was done at 225 nm. |
| *Retention time* | 5.7 min |

The calibration of the system was performed at the same time as the analysis of the extracts. A fixed volume of six different standard solutions covering two orders of magnitude was injected and a calibration graph (peak area versus concentration of analyte in µg/ml) was constructed by using the computer program "Microsoft Excel".

The area arising from the analyte peak was measured and the concentration in the solution was determined from the calibration graph.

Each sample was injected twice. During analysis of the extracts, injections of standard solutions were interspersed with sample injections to provide a continuous check of the instrument calibration.

4.4.6 Analysis of folpet by gas chromatography

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| *Gas chromatograph* | Perkin-Elmer Autosystem |
| *Column* | capillary column DB-1, 50 m x 0.32 mm i.d., 0.24 µm film thickness or SE-54, 30 m x 0.32 mm i.d., 0.25 µm film thickness |
| *Injector* | PTV ("programmed temperature vaporiser"),<br>60 °C, 200 °/min to 320 °C, hold 15 min<br>or 320 °C isothermal<br>injection volume 1 µl<br>splitless (sampling time 2 min) |
| *Carrier gas* | helium at 35 – 40 cm/s |
| *Temperature program* | for DB-1 column: 60 °C, 2 min, 8 °/min to 290 °C, hold 10 min; for SE-54 column: 80 °C, 1 min, 10 °/min to 280 °C |
| *Detector* | $^{63}$Ni electron capture detector (ECD) at 320 ° C, make-up gas nitrogen (65 ml/min) |
| *Retention time* | on DB-1 column 34.9 min<br>on SE-54 column 16.8 min |

## 4.5 Method validation

### 4.5.1 Recovery

The analytical procedure was validated by analysing tap water samples after fortification with folpet at the following concentrations: 0, 0.02, 0.05, 0.1, 0.2, 0.5 and 1.0 µg/l. Each workup of fortified samples was done in duplicate according to the method described in 4.4.

### 4.5.2 Linear range of detector response

A fixed volume of six different standard solutions from 0.004 µg/ml to 0.4 µg/ml was injected and a calibration graph (peak area versus concentration of analyte in µg/ml) was constructed by using the computer program "Microsoft Excel" to determine the linear range of the detector.

### 4.5.3 Precision of injections

The precision of HPLC injections was determined by injecting a sample extract (approx. 0.01 µg/ml) eight times and calculating the standard deviation from the peak areas.

## 4.6 Calculation

### 4.6.1 Calculation of folpet residues

The following equation was used to calculate the residue in the water samples:

$$R = \frac{c \cdot 1000}{V}$$

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| R | residue [µg/l] |
| c | concentration of folpet in the final extract, as calculated from the calibration graph [µg/ml] |
| V | sample volume used for extraction [ml] |

### 4.6.2 Calculation of percentage recovery

The following equation was used for calculation of the percentage recovery from fortification experiments:

$$\%\text{Recovery} = \frac{R}{R_{act}} \cdot 100$$

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| R | residue found after fortification [µg/l] |
| $R_{act}$ | actual residue (fortification level) [µg/l] |

### 4.6.3 Calculation of detection limit and quantification limit

The limits of detection and quantification were calculated from the results of recovery experiments (4.5.1) according to the guidelines of the "Deutsche Forschungsgemeinschaft" (DFG) [3] with the aid of the computer program "KALIBO" (author: J. Vogelgesang, [4]).