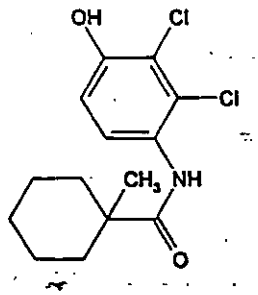
1. Introduction

The method was elaborated for the determination of KBR 2738 in test water from aquatic toxicity tests.

1.1 The active ingredient KBR 2738 is used as fungicide and has the following chemical and physical properties:

Structural formula



Chemical designation	:	N-(2,3-dichloro-4-hydroxy-phenyl)-1-methyl-cyclo-hexanecarboxamide
Empirical formula	:	$C_{14}H_{17}Cl_2NO_2$
Molecular weight	:	302.3 g/mole
Solubility	:	Water 20 mg/l (20°C) Acetone 160 g/l (20°C)

2. Principle of the method

The active ingredient is determined by HPLC with UV-detection. The water samples are directly injected into the HPLC instrument or after appropriate dilution with water.

3. Instruments

Liquid chromatograph : HP 1090 with diode-array-detector
Hewlett Packard Co.,
61352 Bad Homburg, FRG

Comparable instruments of other manufacturers may be used alternatively.

Volumetric flasks, pipettes and other common laboratory equipment.

4. Reagents

Water	:	deionized and cleaned in a Milli-Q-unit
Acetonitrile	:	G-Chromasolv, Merck Co., 64293 Darmstadt, Art. 409930
Sodium dihydrogen-phosphate-2-hydrate	:	Riedel-de-Haën, 30926 Seelze, Art. 04269
Reference substance	:	KBR 2738

A satisfactorily characterized and certified substance is used as reference substance. First a stock solution of about 1000 mg/l in acetonitrile is prepared with the reference substance. The standard solution to be used is prepared by diluting the stock solution with Milli-Q-water.

5. Performance of the analyses

The water samples are injected into the HPLC instrument directly or after appropriate dilution with Milli-Q-water.

Chromatographic conditions

Column	:	Lichrospher Select B, length 125 mm; i.d. 4 mm, Merck Co., 64293 Darmstadt
Particle size	:	5 µm
Oven temperature	:	40°C
Injection volume	:	250 µl*
Flow rate	:	2 ml/min.
Mobile phase	:	Water (with 1 g NaH_2PO_4/l) : acetonitrile, 50:50 (v:v)
Wavelength	:	210 nm
Retention time	:	about 2.0 min.

* The injection volume can be adapted to the concentrations to be measured, if necessary.

6. Evaluation

The evaluation is made by means of a laboratory data system via comparison of the peak areas of the sample with the peak areas of the external standard solutions. The active ingredient content of the sample can be evaluated according to the following formula:

$$R = \frac{Ap \times Cs}{AS}$$

R	=	Active ingredient content of the sample (mg/l)
Ap	=	Peak area of the sample solution (area counts)
As	=	Peak area of the standard solution (area counts)
Cs	=	Concentration of the standard solution (mg/l)

7. Limit of determination

The lower limit of the practical working range of the method is 0.01 mg/l.

8. Linearity

The linearity of the detector was checked for KBR 2738 in the range from 0.01 to 10 mg/l. The resulting curve is represented in Figure 5. The correlation coefficient was 0.99978.

9. Safety Instructions

The German Guidelines for laboratories of the Trade Cooperative Association (e.g. Bulletin M006) or comparable guidelines in other countries must be taken into consideration when working following this method.

The following solvents and plant protectants being classified as toxic and/or low toxic according to the Hazardous Substances Regulation are used. This classification is based on German Guidelines and must be adapted to the respective national guidelines if the method is used outside of Germany.

Toxic: Acetonitrile