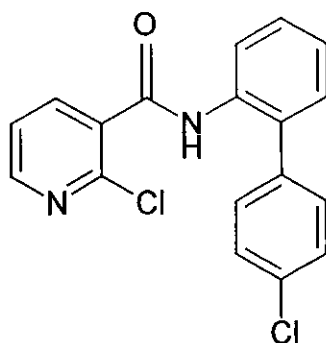


Analytical Method CP-No. 327: The Determination of BAS 510 F in Water**I. PRINCIPLE**

The water samples were diluted with acetonitrile, followed by dilution with an acetonitrile: freshwater algal medium (50:50, v/v) and then directly analyzed by HPLC using UV detector at wavelength of 233 nm to determine the residues of BAS 510 F in water. The method allows the determination of BAS 510 F with the required limit of quantitation (0.2 ppm) in water.

II. MATERIALS/METHODS**A. TEST AND REFERENCE SUBSTANCE****Fortification Compounds and Reference Standards (used for calibration):**

BASF Code Name:	BAS 510 F
BASF Registry Number:	300355
Chemical Name:	2-Chloro-N-(4'-chlorobiphenyl-2-yl)nicotinamide
Molecular Formula:	$C_{18}H_{12}Cl_2N_2O$
Molecular Weight:	343.21
Appearance:	White powder
Water Solubility:	4.63 mg/L
Lot No.:	01183-190
Purity:	99.3%
Structural Formula:	



Standard substances are stored in a freezer (<-5°C) until use. Characterization, purity and stability were determined prior to use for this study. Details of these determinations are available to BASF and are located at Landwirtschaftliche Versuchsstation der BASF, Limburgerhof, Germany.

Test and reference substance solutions were refrigerated during their use in this study. Stock solutions (1 mg/mL) were made fresh every three months and further diluted to proper concentration. Dilutions of stock standards for

II. MATERIALS/METHODS (Continued)

fortifications were made fresh every month. During the course of this study, the stability of fortification and HPLC-UV standard solutions were examined. Solutions were stored in a refrigerator at 4°C.

B. TEST SYSTEM

The Freshwater Algal Medium was used as test system

C. SAMPLE STORAGE AND HANDLING

The water samples were stored under ambient conditions before analysis.

D. EXPERIMENTAL DESIGN

To determine recoveries of BAS 510 control water samples were fortified by applying standard solutions directly to the water prior to the analysis. Samples were fortified with 0.2, 1.0 and 4.0 ppm of BAS 510 F and subsequently analyzed with the method. At least one fortification level consisting of two replicates was analyzed. Analyses of six samples were conducted.

E. METHOD OF ANALYSIS

BASF Analytical Method CP-No. 327 was used to determine the residues of BAS 510 F in the freshwater alga matrices using HPLC-UV. The method was designed to determine the residues as an individual analytes and was used in studies to determine the toxicity of BAS 510 F to the freshwater alga (*Anabaena flos-aquae*) during a 96-hour exposure period under static test conditions.

E.1 ANALYTICAL STOCK/STANDARDS PREPARATION

A stock solution of BAS 510 F was prepared by accurately weighing 0.1036 g (corrected for purity) of the test substance on an analytical balance. The test substance was transferred to a 100-mL class A volumetric flask, and brought to volume using acetonitrile. This primary stock solution contained 1.00 µg a.i./µL of BAS 510 F. The resulting stock (1.00 µg a.i./µL) was diluted in acetonitrile to prepare a 0.100 µg a.i./µL secondary stock. The secondary stock solution was used to fortify the quality control samples. A stock solution of BAS 510 F analytical standard was prepared by accurately weighing 0.02521 g (corrected for purity) of the analytical standard on an analytical balance. The substance was transferred to a 50-mL class A volumetric flask, and brought to volume using acetonitrile. This primary stock solution contained 0.501 µg a.i./µL of BAS 510 F.

The resulting stock (0.501 µg a.i./µL) was diluted in acetonitrile to prepare a 0.100 µg a.i./µL secondary stock. The following shows the dilution scheme for the set of calibration standards.

Stock Concentration (µg a.i./ µL)	Aliquot (µL)	Final Volume (mL)	Standard Concentration (mg a.i./L)
0.100	60.0	100	0.060
0.100	150	100	0.150
0.100	250	100	0.250
0.100	350	100	0.350
0.100	600	100	0.600

E.2 ANALYTICAL METHOD

The method used for the analysis of BAS 510 F in freshwater algal medium samples was based on BASF Method CP-No. 327 that was initially developed (Reference 1) to determine the residues of BAS 510 F in water using HPLC with UV detection at BASF Akitiengesellschaft, Limburgerhof, Germany. Samples were processed and analyzed as follows:

The analytical method consisted of diluting all samples in acetonitrile (CH₃CN). Secondary dilutions were made, as necessary, in 50:50, CH₃CN-freshwater algal medium (v/v).

Freshwater algal medium samples were fortified at 0.200, 1.00 and 4.00 mg a.i./L and analyzed concurrently with the samples to determine the mean procedural recovery (Table I). Reported measured concentrations for the samples were not corrected for the mean procedural recovery of 103%.

Concentrations of BAS 510 F were determined by high performance liquid chromatography using a Hewlett-Packard Model 1090 High Performance Liquid Chromatograph (HPLC) equipped with a Hewlett-Packard Model 1100 Variable Wavelength Detector (VWD) or a Waters 486 Variable Wavelength Detector. HPLC separations were achieved using a Zorbax phenyl analytical column (250 mm x 4.6 mm, 5-µm particle size).

Calibration standards of the BAS 510F prepared 50:50, CH₃CN-freshwater algal medium (v/v), ranging in concentration from 0.06 to 0.6 mg a.i./L, was analyzed with each sample set.

Linear regression equations were generated using the peak area responses versus the respective concentrations of the calibration standards. The concentration of BAS 510 F in the samples was determined by substituting the area responses into the applicable linear regression equation.

The method limit of detection (LOD) for the analyses was set at 0.120 mg a.i./L, calculated as the product of the lowest calibration standard (0.0600 mg a.i./L) and the dilution factor of the matrix blank samples (2.00) analyzed concurrently with the test samples. Two matrix blank samples were analyzed to determine possible interference. No interference was observed at or above the LOD during the sample analyses.

A method flowchart is provided in **Figure 1**.

The specific chromatographic conditions and instrumental parameters are summarized in **Figure 2**

Typical recovery calculations for the HPLC-UV quantitation are shown in **Figure 3**.

Typical standard curves and chromatograms of standard, and soil samples are provided in **Figures 4.1 through 4.3**.

Figure 1. Analytical Method Flowchart for the Analysis of BAS 510 F in Freshwater Algal Medium

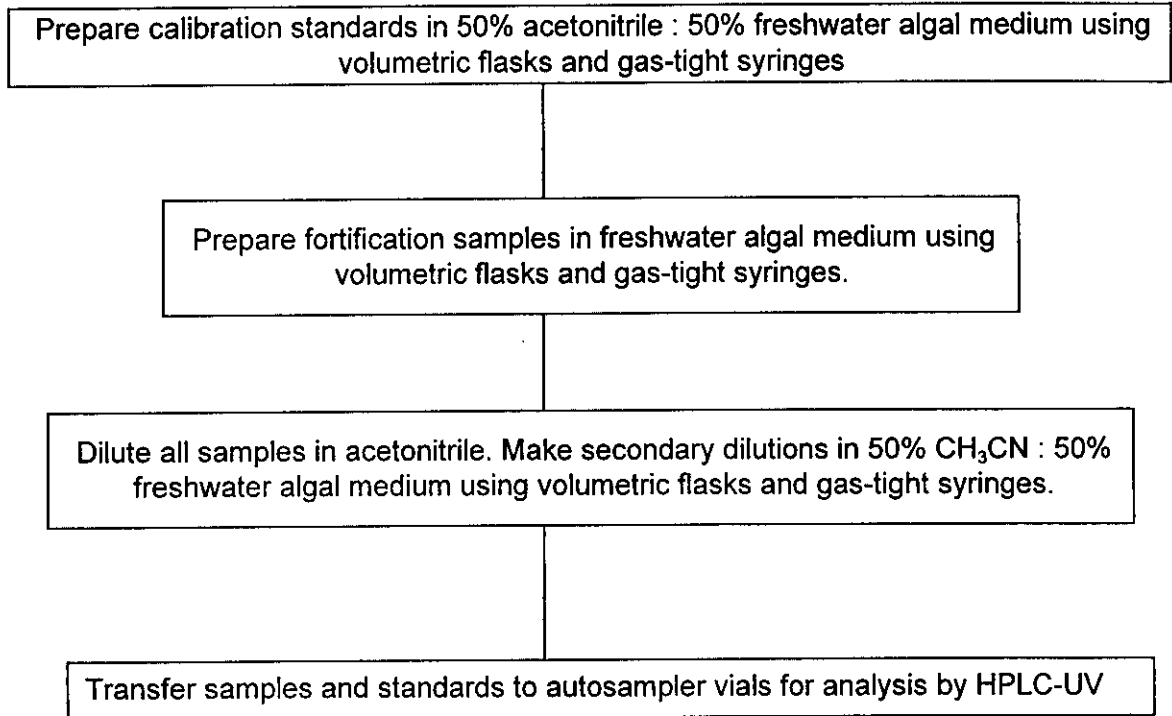


Figure 2. Typical HPLC Operational Parameters

INSTRUMENT:	Hewlett-Packard Model 1090 High Performance Liquid Chromatograph (HPLC) Equipped with a Hewlett-Packard Model 1100 Variable Wavelength Detector (VWD) or a Waters 486 Variable Wavelength Detector			
ANALYTICAL COLUMN:	Zorbax phenyl (250 mm x 4.6 mm, 5 µm particle size)			
FLOW RATE:	1.0 mL/minute			
OVEN TEMPERATURE:	40°C			
STOP TIME:	15.0 minutes			
SOLVENT A:	10% CH ₃ CN ; 90% H ₂ O : 0.1% H ₃ PO ₄			
SOLVENT B:	95% CH ₃ CN ; 5% H ₂ O : 0.1% H ₃ PO ₄			
GRADIENT PROFILE:	Time (Minutes)	% A	% B	Flow (mL/min)
	0.01	60	40	1.0
	1.00	60	40	1.000
	8.00	0	100	1.000
	10.00	0	100	1.000
	10.10	60	40	1.000
	15.00	60	40	1.000
INJECTION VOLUME:	100 µL			
BAS 510 F PEAK RETENTION TIME:	Approximately 9.1 minutes			
ANALYTICAL WAVELENGTH:	233 nm			

Figure 3: Typical Recovery Calculation

Example Calculations for a Representative Sample:

The analytical result and percent recovery for sample number 147A-178-5 (obtained from study 46667, Reference 2), with a nominal concentration of 1.0 mg a.i./L, was calculated using the following equations:

$$\text{Concentration of BAS 510 F in sample (mg a.i./L)} = \frac{\text{peak area} - (\text{y-intercept}) \times \text{dilution factor}}{\text{slope}}$$

$$\text{Percent of nominal concentration} = \frac{\text{Measured concentration of sample (mg a.i./L)}}{\text{Nominal concentration of sample (mg a.i./L)}} \times 100$$

$$\text{Peak Area} = 174.01053$$

$$\text{Y-Intercept} = 0.8266$$

$$\text{Slope} = 345.5101$$

$$\text{Initial Volume (Vi)} = 1.00 \text{ mL}$$

$$\text{Final Volume (Vf)} = 2.00 \text{ mL}$$

$$\text{Dilution Factor (Vf/Vi)} = 2.00$$

$$\text{Concentration of BAS 510 F in sample (mg a.i./L)} = \frac{174.01053 - 0.8266 \times 2.00}{345.5101}$$

$$\text{Concentration of BAS 510 F in sample (mg a.i./L)} = 1.00$$

$$\text{Percent of nominal concentration} = \frac{1.00 \text{ mg a.i./L}}{1.0 \text{ mg a.i./L}} \times 100$$

$$\text{Percent of nominal concentration} = 100\%$$