Independent Laboratory Validation of BASF Analytical Method D1706/01: "Method for the determination of BAS 510 F (Reg. No. 300355) in sediment by LC-MS/MS"

ABSTRACT

The purpose of this study was to demonstrate the performance of BASF Analytical Method D1706/01: "Method for the determination of BAS 510 F (Reg. No. 300355) in sediment by LC-MS/MS" with acceptable recoveries at an outside laboratory with no prior experience with the method. This method was originally developed and validated at BASF Crop Protection, RTP, NC.

Principle of the Method: In sediments, residue of BAS 510 F was extracted by shaking with methanol/0.098 M sodium acetate (aq)/0.102 M acetic acid (aq), 80/10/10, v/v/v followed by centrifuging a 10 mL aliquot. A 1:10 dilution was performed on the centrifuged extract by mixing 0.1 mL of the extract with 0.9 mL of deionized (DI) water. Final determinations were conducted using LC-MS/MS in the positive ion mode.

The final determination of BAS 510 F was performed by LC-MS/MS. For BAS 510 F, the transition at *m/z* 343→307 was monitored in positive mode for primary quantification; the transition at *m/z* 343→271 was monitored in positive mode for confirmation.

Test Conditions: The method was independently validated at two fortification levels. The fortification levels were 0.005 and 0.05 mg/kg (ppm) for BAS 510 F. For each fortification level, five replicates were analysed. Additionally, a reagent blank and two replicates of unfortified samples were examined.

Limit of Quantitation (LOQ) and Limit of Detection (LOD): The LOQ was defined as the lowest fortification level tested. The LOQ for BAS 510 F in sediments was 0.005 mg/kg (ppm). The LOD for BAS 510 F in sediments was set at 0.001 mg/kg (ppm), which was 20% of the defined LOQ.

Selectivity: The method determines residues of BAS 510 F in sediments. No interfering peaks were found at the retention times of BAS 510 F. It was determined that matrix match standards were not needed. The MRM transitions used to identify BAS 510 F were adapted from method D1706/01 and refined and/or adjusted to the specific instrument used during this study.

Linearity: Good linearity ($r^2 < 0.99$) was observed in the standard calibration solutions in the range of 0.01 ng/mL to 1 ng/mL for all the mass transitions for BAS 510 F.

Standard Stability: Standard stability was determined in method validation study (Reference 1). The stock solution and fortification solutions of BAS 510 F prepared in methanol were stable for 118 days at both room temperature and under refrigeration. Dilutions of the stock solution, fortification and calibration solutions were determined to be stable for up to 1 month when stored refrigerated.

Extract Stability: Extract stability was established in the method validation study.

1. Introduction

1.1 Scope of the Method

BASF method D1706/01 was developed to determine the residues of BAS 510 F in sediments by LC-MS/MS. This method was developed and validated at BASF Crop Protection in Research Triangle Park, North Carolina (Reference 1) and was independently validated at EPL Bio Analytical Services.

The independent lab validation was conducted using two fortification levels for BAS 510 F in sediments. In Golden Lake sediment and Goose River sediment matrices, the fortification levels were 0.005 mg/kg (ppm) and 0.05 mg/kg (ppm). For each fortification level, five replicates were analyzed. Additionally, one reagent blank and two replicates of unfortified samples were examined.

1.2 Principle of the Method

In sediments, residue of BAS 510 F was extracted by shaking with methanol/sodium acetate buffer. A 1:10 dilution was performed with DI water. Final determinations were conducted using LC-MS/MS in the positive ion mode.

The final determination of BAS 510 F was performed by LC-MS/MS. For BAS 510 F, the transition at m/z 343→307 was monitored in positive mode for primary quantification; the transition at m/z 343→271 was monitored in positive mode for confirmation.

1.3 Specificity

To demonstrate the specificity of the analytical method, one additional mass transition was monitored simultaneous to the primary detection transitions for BAS 510 F. The method was able to accurately determine residues of BAS 510 F, and no interference was observed at the retention time for the analyte peak in sediments.

2. Materials and Methods

2.1 Test System

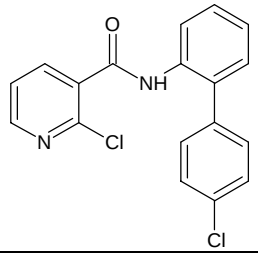
The test systems considered in this study were Golden Lake sediment and Goose River sediment.

The control samples were provided by BASF. The Golden Lake sediment and Goose River sediment control samples were received on August 3, 2017. Upon arrival at the laboratory, the samples were opened, inspected, and checked against enclosed shipping forms. The test systems were stored refrigerated at all times, unless necessary for laboratory analysis.

2.2 Test/Reference Substances

The BAS 510 F analytical reference substance was provided by the sponsor and was received on August 2, 2017. BASF has retained reserve sample of the chemical, and has documentation specifying the location of the synthesis and characterization information for the compound available at BASF Crop Protection, Research Triangle Park, North Carolina. The analytical reference substance was stored refrigerated when not in use. The certificate of analysis is presented in Appendix D. Summary of the reference substance is presented below.

BAS 510 F

Common Name	Boscalid
BASF Reg. No.	300355
CAS No.	188425-85-6
Molecular Formula	$C_{18}H_{12}Cl_2N_2O$
Molecular Weight	343.2 g/mol
IUPAC Name	2-chloro-N-(4'-chlorobiphenyl-2-yl)nicotinamide
Batch Number	L71-168
Purity	99.0 %
Storage	Refrigerated
Expiration Date	March 01, 2020
Chemical Structure	

2.3 Materials

2.3.1 Equipment

The equipment used in this study was documented in the raw data. Maintenance files and applicable SOPs for the equipment are retained at the testing facility.

Class A volumetric glassware
Laboratory glassware (beakers, flasks, graduated cylinders, scintillation vials)
Erlenmeyer Flask (250 mL)
Glass culture tubes
Volumetric pipettes, glass; various sizes
Analytical balance
Top loader balance
Air displacement pipette, various volumes with disposable tips
Mechanical shaker
Centrifuge, with rotors to accommodate 15 mL centrifuge tubes or culture tubes
Vortex mixer
0.45 µm PTFE filter and syringes
Sonicator
HPLC system: Agilent 1290
HPLC analytical column: (Waters) BEH C18, 2.1 x 50 mm (1.7 µm)
Mass spectrometer: AB Sciex 6500 Q-Trap
HPLC autosampler vials with screw-top, pre-slit caps

2.3.2 Reagents

Chemicals

Chemical	Manufacturer/ Supplier	Lot Number	Expiration Date
Sodium Acetate	JT Baker	139685	9/12/2019
Acetic Acid	BDH	2015080565	6/30/2020
Formic Acid	Sigma-Aldrich	SHBH8394	1/31/2018
Methanol	EMD	56204	6/30/2021
Deionized (DI) Water	EPL in-house DI water system	N/A	N/A

N/A = Not Applicable

Solutions and Solvent Mixtures

Description	Code	Composition
0.2 M Sodium acetate (aq)	S1	0.2 M Sodium acetate (aq)
0.2 M Acetic acid (aq)	S2	0.2 M Acetic acid (aq)
Sodium acetate buffer (pH = 4.6)	S3	0.098 M sodium acetate (aq)/0.102 M acetic acid (aq), 49/51 v/v
Extraction solvent	S4	Methanol/0.098 M sodium acetate (aq)/0.102 M acetic acid (aq), 80/10/10, v/v/v
Final Volume Solvent	FV1	Methanol/9.8 mM sodium acetate (aq)/10.2 mM acetic acid (aq)/H_2O 8/1/1/90, v/v/v/v
HPLC mobile phase A	LC1	0.1% Formic Acid in Water
HPLC mobile phase B	LC2	0.1% Formic Acid in Methanol

2.3.3 Standard Solutions

Example Preparation of Stock Solution

Individual 1.0 mg/mL stock solution of BAS 510 F was prepared by weighing 0.01 g of BAS 510 F into a 10 mL volumetric flask and bringing to volume with methanol. A homogenous solution was obtained through vortexing and sonication. Fortification and calibration standard solutions were prepared in separate dilution series from the same stock.

ID	Purity	Weight (g)	Concentration (µg/mL)
BAS 510 F	99.0%	0.01006	1006.000

Example Preparation of Fortification Solution and Working Standards

Fortification and calibration standard solutions were prepared from one stock solution in separate dilution series. Two standard solutions of 10 µg/mL were prepared by transferring 0.5 mL of the BAS 510 F stock solution into a 50 mL volumetric flask and diluting to volume with methanol first. From the two standard solutions, two dilution series were prepared to make fortification solutions and working standard solutions respectively, as exemplified in the tables below. The use of sonication or vortexing was also considered for ensuring a complete homogeneous solution.

Example Preparation of Fortification Solutions – BAS 510 F

Solution Used (µg/mL)	Volume Taken (mL)	Diluted with methanol to a final volume of (mL)	Concentration (µg/mL)
1006.000	0.5	50	10.060
10.060	5	50	1.006
1.006	5	50	0.101

Example Preparation of Calibration Standard Solutions

Standard calibration solutions were prepared using one of the working standard solutions prepared in the previous section and diluting with methanol/9.8 mM sodium acetate (aq)/10.2 mM acetic acid (aq)/H_2O (8/1/1/90, v/v/v/v) as needed. These solutions were prepared according to the tables below.

Example Preparation of Standard Solutions for Calibration

Take solution (ng/mL)	Volume (mL)	Dilute with FV1 to a final volume of (mL)	Concentration (ng/mL)
101.000	1	100	1.010
1.010	50	100	0.505
0.505	20	50	0.202
0.505	5	50	0.0505
0.505	2.5	50	0.0253
0.505	1	50	0.0101

3. Analytical Procedure

3.1 Weighing and Fortification

5 g (4.9 – 5.1 g) of sediment sample was weighed into a 250 mL Erlenmeyer flask. Fortification samples were spiked with the appropriate standard solution to obtain five fortifications at the LOQ (0.005 ppm) and five fortifications at 10 x LOQ (0.05 ppm). The following scheme was used:

Sample Type	Sample Weight	Concentration of Spiking Solution	Volume of Spiking Solution	Level of Fortification
Control	5 g	-	-	-
Fortification (LOQ)	5 g	1 µg/mL	0.025 mL	0.005 mg/kg
Fortification (10x LOQ)	5 g	10 µg /mL	0.025 mL	0.05 mg/kg

3.2 Extraction of Sample Material

50 mL of extraction solvent methanol/0.098 M sodium acetate (aq)/0.102 M acetic acid (aq), (80/10/10, v/v/v) was added and the samples were placed on a mechanical shaker for 60 minutes at 300 rpm. Approximately 10 mL aliquots were transferred to culture tubes and centrifuged at 3500 rpm for 5 min.

3.3 Preparation for Measurement

0.1 mL of sample extracts were transferred to culture tubes and were diluted to 1 mL with DI water. Samples were filtered through a 0.45 µm PTFE filter and transferred to LC vials for analysis.

4. Instrumentation and Conditions

	Parameter		
Chromatographic System	Agilent 1290		
Analytical-column	Acquity BEH C18, 2.1 x 50 mm, 1.7 µm		
Column Temperature	50°C		
Injection Volume	40 µL		
Mobile Phase A Mobile Phase B	A = 0.1% Formic Acid in Water B = 0.1% Formic Acid in Methanol		
Flow Rate	600 µL/min		
Gradient	Time (min)	Phase A (%)	Phase B (%)
	0.00	75	25
	0.05	75	25
	0.90	55	45
	1.50	5	95
	2.45	5	95
	2.50	75	25
	3.00	75	25
Detection System	AB Sciex 6500 Mass Spectrometer		
Ionization	Electrospray (ESI)		
Ionization Temperature	750 °C		
Analyte	**Transitions**	**Polarity**	**Expected Retention Time**
BAS 510 F	342.8 --> 307.2* 342.8 --> 270.9	positive	approx. 1.95 min

* Primary quantification transition.

4.1 Calibration Procedures

Calculation of results was based on peak area measurements using a linear calibration curve (weighted 1/x). The calibration curve for BAS 510 F was obtained by direct injection of external calibration standards containing BAS 510 F in the range of 0.01 ng/mL to 1 ng/mL.

4.2 Rounding of Numbers

Numerical values in this report are frequently rounded to a smaller degree of precision (number of digits) than were used in the actual calculation to increase readability and to indicate the approximate precision of the reported results. Minor differences in the results obtained with such “rounded” values in comparison to those obtained with higher precision values are well within the limits of the experimental accuracy and therefore of no practical concern.

4.3 Statistical Analysis of Data

Mean recoveries were calculated on the data generated where appropriate. Full computer/calculator precision was used in any intermediate calculations, and only the final value was rounded. Slight differences may be noted in hand calculations versus calculations in the individual data tables presented in this report due to rounding and significant figures presented in calibration curve data provided by the mass spectroscopy instrumentation. Simple descriptive statistics were performed on the data (average, standard deviation, and relative standard deviation), as considered appropriate. Statistical treatment of the data included simple descriptive statistics, such as determinations of averages for the procedural recoveries and area counts, and calculation of the calibration curve and coefficient of variation (r) by linear regression of the instrument responses for the analytical reference standards.

4.4 Calculation of Residues and Recoveries

Data was acquired with validated Analyst software. The data processing was completed in MultiQuant, which is a companion software program accessed via Analyst.

The residue content was calculated following method D1706/01:

$$\text{Residue [mg/kg]} = \frac{V_{end} \times C_A}{G \times A_F \times 1000}$$

V_{end} = Final volume of the extract after all dilution steps [mL]

C_A = Concentration of analyte as read from the calibration curve [ng/mL]

G = Weight of the sample extracted [g]

A_F = Aliquot factor

1000 = Factor remaining after all unit conversions

The Aliquot Factor and Final Volume were therefore calculated as:

As the final volume (V_{end}) is 1 mL as listed in the method section 3.5:

$$A_F = \frac{0.1\,(\text{mL})}{50\,(\text{mL}) * 1} = 0.002$$

V_{end} = 1 mL

Nominal Sample Concentration (ppm, mg/kg) =

$$\frac{V_{end}\,(\text{mL}) * \text{Nominal Std Conc. (ng/mL)}}{\text{Aliquot Factor (mL/mg)} * \text{Ideal Sample Weight (5.0 g)} * 1000}$$

Response Factor (no units expressed) =

$$\frac{\text{Peak Area (cps)}}{\text{Nominal Standard Concentration (ng/mL)}}$$

ppm Found =

$$\frac{[\text{Sample Analytical Result (ng/mL)} - \text{Blank Analytical Result (ng/mL)}] * \text{Vend (mL)}}{\text{Aliquot Factor} * \text{Sample Weight (g)} * 1000 \text{ (ng/mg)}}$$

Relative Error Accuracy (%) =

$$\frac{(\text{Calculated Standard Concentration (ng/mL)} - \text{Nominal Standard Concentration (ng/mL)})}{\text{Nominal Standard Concentration (ng/mL)}} * 100$$

Fortification Level (ppm) =

$$\frac{\text{Volume Spiking Solution (mL)} * \text{Concentration of Spiking Solution (µg/mL)}}{\text{Sample Weight (g)}}$$

Recovery (%) =

$$\frac{\text{ppm Found in Spike} * 100}{\text{Fortification Level (ppm)}}$$

Relative Standard Deviation (%) =

$$\frac{\text{Standard Deviation} * 100}{\text{Average Recoveries}}$$

5. Results

5.1 Validation Data

According to the method D1706/01, sediment matrices including Golden Lake sediment and Goose River sediment were fortified with a solution containing BAS 510 F to obtain concentrations of 0.005 mg/kg (LOQ) and 0.05 mg/kg (10x LOQ), respectively.

To test the repeatability of the method, the sample replicates were divided into the following sets for analysis: one method blank, two unfortified control samples, and five fortifications at both the LOQ and 10x LOQ fortification level.

Control samples were treated in exactly the same way as fortified samples. All results obtained from the measurement of control samples were below the set LOD for BAS 510 F. Therefore, no interferences in the control samples could be determined.

Quantification was done by LC-MS/MS according to method D1706/01 for sediment matrices. A summary of all recovery data for each fortification level is presented in Table 1. Detailed analytical tables are presented in Appendix C.

5.2 Summary of Method

Type of Method	LC-MS/MS
Test Systems	Golden Lake sediment, Goose River sediment
Selected mass transitions (m/z)	BAS 510 F 342.8→307.2* 342.8→270.9 *Primary quantification transition
Analytical Procedure	BASF Analytical Method D1706/01: "Method for the determination of BAS 510 F (Reg. No. 300355) in sediment by LC-MS/MS"
Confirmatory Technique	Due to the high selectivity and specificity of LC-MS/MS, an additional confirmatory technique was not necessary. A secondary MRM transition was used for confirmation.
Method of Quantitation	The quantitation was based on the monitoring of two mass transitions for BAS 510 F. External calibration standards were used to calculate the recoveries as matrix effects had no influence on sample analysis during method validation. Recovery data was reported for each mass transition considered, as shown in Appendices A-B.
LOD	0.001 mg/kg (ppm) for BAS 510 F in sediments
LOQ	0.005 mg/kg (ppm) for BAS 510 F in sediments
Levels of Fortification	0.005 and 0.05 mg/kg (ppm) (sediments)
Time Required	A set of 13 samples requires approximately 4-8 hours of work.