Abstract

The objective of this validation study was to demonstrate the applicability and repeatability of BASF Analytical Method D1706/01 for the determination of BAS 510 F in sediment matrices by using LC-MS/MS.

Principle of the method. Residues of BAS 510 F in sediment samples is extracted by shaking with methanol/sodium acetate(aq) buffer. The extract is then diluted with water for analysis by a liquid chromatography (LC) column with detection by positive ion electrospray ionization tandem mass spectrometry (ESI-MS/MS) monitoring the following ion transitions: m/z 343→307 and 343→271 for BAS 510 F.The results are calculated by direct comparison of the sample peak responses to those of external standards.

Test conditions. For validation, untreated goose river and golden lake sediment samples were fortified with BAS 510 F and analyzed according to the established method validation guidelines. The analytical sets for each sediment type typically consisted of a reagent blank, two controls, five replicates fortified with analyte at the method limit of quantitation, 0.005 mg/kg (ppm) for BAS 510 F, and five replicates fortified at a higher level, corresponding to 10X the limit of quantitation, 0.05 mg/kg for BAS 510 F. The two mass transitions described above for BAS 510 F were evaluated via LC-MS/MS procedures. In conjunction with the subject study, matrix- and solvent-matched standards were analyzed to evaluate any potential matrix effects.

Limit of Quantification (LOQ) and Limit of Detection (LOD). The LOQ of the method was set at 0.005 mg/kg for BAS 510 F, covers the lowest relevant eco-toxicology endpoint in sediment and/or relevant enivornmental compartment. The LOD for BAS 510 F was set at 0.001 mg/kg, which is 20% of LOQ.

Selectivity. The method determines BAS 510 F residues in sediment matrices by LC-MS/MS. No interfering peaks were found at the retention times for these analytes. The multiple reaction monitoring (MRM) transitions used to identify BAS 510 F were determined by product ion spectra. The experiment to evaluate any potential matrix effects showed that the matrix load in the samples from each sediment type had no significant influence on analysis (matrix effects <20%).

Linearity. Acceptable linearity was observed for the standard range tested for each analyte: The method-detector response, for the method validation sets, was linear over the 0.01 to 0.5 ng/mL range for parent ($r \geq 0.9991$).

Standard stability. The stability of BAS 510 F in standard solutions has been determined: In this study, BAS 510 F was shown to be stable in calibration standard solutions prepared by serial dilution of the intermediate standards with methanol/sodium acetate(aq)/acetic acid(aq)/water (8/1/1/90 v/v ratio) and held under refrigeration for at least 30 days. Stability of BAS 510 F in methanol for stock/fortification solutions was determonstrated for 118 days in another study. A summary of the stability of BAS 510 F in standard solutions is provided in Appendix E. During the course of this study, all solutions were held under refrigeration and all solutions were used within the demonstrated time period of stability.

1 Introduction

1.1 Background and Purpose of Study

The objective of this validation study was to demonstrate the applicability and repeatability of BASF Analytical Method No. D1706/01, used for the determination of residues of BAS 510 F in sediment by LC-MS/MS.

2 Materials and Methods

2.1 Test Systems

The sediment samples used in this study were Goose river sediment and Golden lake sediment samples, which were characterized by AGVISE Laboratories. The GLP sediment characterization reports are provided in Appendix K. The samples were refrigerated during the experimental period. Each analysis set was uniquely identified with a Master Sheet Number, which consisted of the study number plus a unique number (e.g., 828758-1-1). The test system samples were assigned unique numbers and these were recorded in each analytical set or "Master Sheet" (e.g., sediment fortification sample 828758-2-4 or 0.005 PPM, from Master Sheet No. 828758-2 using control matrix sample CM17-025). The actual sample numbers used for the analysis were identified in the raw data and in this final report.

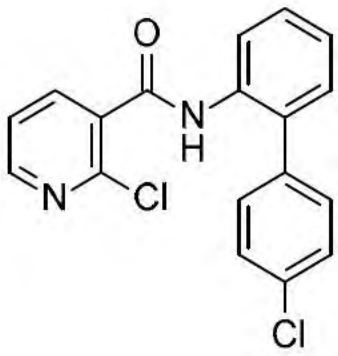
2.2 Test and Reference Substances

The test/reference standards shown below were synthesized by BASF Aktiengesellschaft (Limburgerhof, Germany) and used during the analytical portion of this study. The test/reference substances were maintained according to the storage advice reflected on the certificates of analysis until use in this study. BASF Aktiengesellschaft determined characterization and purity prior to the substance being used in this study. Details of these determinations are available to BASF and are located at Landwirtschaftliche Versuchsstation der BASF, Limburgerhof, Germany. The "Certificate of Analysis" for each test/reference standard is shown in Appendix A.

The test/reference substances in solution were used in the study to generate data for both instrument and method performance. Quantitation of residues in all samples was achieved using calibration curves calculated by linear regression (1/x weighting) of instrument responses for the reference substances. The performance of the instrument was evaluated during each injection set.

2.2.1 BAS 510 F

Internal-Code	BAS 510 F
Common Name	Boscalid
IUPAC Name	2-Chloro-*N*-(4'-chlorobiphenyl-2-yl)nicotinamide
BASF Reg. No.	300355
CAS-No.	188425-85-6
Molecular Formula	$C_{18}H_{12}Cl_2N_2O$
Molecular Weight	343.21
Lot Number	L71-168
Purity	99.0%
Expiration Date	March 01, 2020



Stock solutions of analytes were prepared in methanol. The mixed intermediate/fortification solutions containing each analyte were prepared by combining aliquots of the stock solutions for each analyte and diluting with methanol. The calibration standards were prepared by serial dilution of the intermediate standards using methanol/9.8mM sodium acetate(aq)/10.2mM acetic acid(aq)/water (8/1/1/90 v/v/v/v). The stability of BAS 510 F in calibration standard solution was determined in this study by analyzing aged calibration standards against freshly prepared calibration standard solutions from the same stock. The stability of parent in stock/fortification solutions has been determined in a related study (reference 1).

During the course of this study, the test/reference substance solutions were stored under refrigeration. Preparation and dilution data forms pertaining to the stock and working solutions are located in the analytical facility data and are archived periodically. Example standard dilution and use information, as performed in the subject study, are provided in Appendix L.

2.3 Route of Administration

In this method validation study, the test substances were applied to the test system as analytical standard solutions (in methanol) by pipette to ensure precise delivery of a small amount of the test substances.

2.4 Analytical Method

2.4.1 Principle of the Method

Using BASF Analytical Method No. D1706/01, residues of BAS 510 F in sediment are quantified using LC-MS/MS. The method procedures validated in this study are provided in Appendix B. A description of the methodology follows: Briefly, residues in sediment samples (5 g) are extracted with 50 mL methanol/sodium acetate buffer and shaken for 60 min on a mechanical shaker at 300 rpm. The extracts are then diluted with water and filtered for analyzsis by LC-MS/MS.

2.4.2 Specificity/Selectivity

Residues of BAS 510 F are determined by LC-MS/MS, in positive mode, monitoring the following ion transitions: m/z 343→307 and 343→271 . The results are calculated by direct comparison of the sample peak responses to those of external standards. Two mass transitions are available for all analytes. Due to the high selectivity and specificity of LC-MS/MS an

additional confirmatory technique is not necessary. The multiple reaction monitoring (MRM) transitions used to identify BAS 510 F were determined in this study.

2.5 Validation of Method

For validation, untreated Goose river sediment and Golden lake sediment samples were fortified with BAS 510 F and analyzed according to the established method validation guidelines. To test the repeatability of the method, the analytical sets consisted of a reagent blank, and for each matrix, two controls, five replicates fortified with each analyte at the method limit of quantitation, 0.005 mg/kg for BAS 510 F and five replicates fortified at a higher level, corresponding to 10X the limit of quantitation, 0.05 mg/kg. For BAS 510 F, the two mass transitions described above were evaluated.

2.6 Influence of Matrix Effects on Analysis

In conjunction with the subject study, matrix-matched standards and solvent-based standards were analyzed to evaluate any potential matrix effects on LC/MS/MS analysis. This involved comparing calibration standards prepared in control matrix against calibration standard solutions prepared with methanol/sodium acetate buffer. The matrix-matched standards were prepared by diluting standards containing BAS 510 F with control goose river or golden lake sediment extract to approximated final concentation of 0.025 ng/mL, 0.05 ng/mL, and 0.2 ng/mL. These standards were then compared to solvent-based calibration standards. Each set of matrix-matched standards was bracketed by a block of solvent-based calibration standards and included additional single injections of the tested standard levels during the run.

The data generated were evaluated by comparing the average area response of the standards for three or more injections of each type (with and without matrix) for the three standard concentration levels. Acceptability (i.e., matrices had no significant influence on the analysis) requires a difference in area of <20% from expected concentration, calculated as the "Mean Area Change (%)". For each matrix, an overall average "Mean Area Change (%)" across the three tested concentrations was calculated to make a general assessment of acceptability with respect to matrix effects.

2.7 Stability of Extracts

The stability of BAS 510 F in extracts was established for each matrix by reanalyzing control and five recovery samples that had been stored under refrigeration prior to dilution and preparation of analysis.The stability in the final volume, the solution prepared for LC-MS/MS injection, was established for each matrix by reanalyzing a control and five recovery samples which had been stored under refrigeration at the final volume stage. Quantification of the analytes in the stored samples for this experiment was performed for the primary mass transitions.

3.2 Influence of Matrix Effects

In conjunction with the subject study, matrix-matched standards and solvent-based standards were analyzed to evaluate any potential matrix effects on LC-MS/MS analysis. The results on each sediment type demonstrated that the matrix load in the samples had no significant influence on the analysis. Therefore, the validation samples were analyzed using solvent-based calibration standard solutions. Matrix effects, calculated as the overall mean percent area count difference between matrix-matched standards and solvent-based standards, at three standard concentration levels, were less than 20% (Appendix G).

3.3 Storage Stability of Solutions

Standards. Stock and fortification solutions of BAS 510 F are prepared in methanol, and calibration standards are prepared by serial dilution of the intermediate standards using methanol/9.8mM sodium acetate(aq)/10.2mM acetic acid(aq)/water (8/1/1/90 v/v/v/v). BAS 510 F was shown to be stable in calibration standard solutions prepared by serial dilution of the intermediate standards with methanol/9.8mM sodium acetate(aq)/10.2mM acetic acid(aq)/water (8/1/1/90 v/v/v/v) and held under refrigeration for at least 30 days. Stability of BAS 510 F in Methanol for stock/fortification solutions was determined in another study (Reference 1). A summary of the stability of the analyte in standard solutions is provided in Appendix E. During the course of this study, all solutions were held under refrigeration and all solutions were used within the demonstrated time period of stability.

Extracts. The method validation fortification sample extracts were analyzed within 3 days of extraction. The generally acceptable method recoveries obtained during analyses demonstrate the storage stability of residues of BAS 510 F in the extracts in the brief period prior to analysis. In addition, the recoveries from stored solutions generated during extract stability experiments performed in conjunction with this study, which included tests on the LC final volume held under refrigeration, indicated the analyte, for both sediment types tested is stable in extracts for at least 6 days as shown in Appendix D.

4 CALCULATIONS AND RAW DATA

An example calculation is included in Appendix C. Detailed analytical data such as supporting raw data necessary for re-calculations, standards and calibration curve data are provided in Appendix F. Example standard curves are provided in Appendix H. Example chromatograms are provided in **Appendix I**.

5 STATISTICS AND DATA INTEGRITY

Statistical treatment of the data included simple descriptive statistics, such as determinations of averages, standard deviation and/or RSD for the procedural recoveries and area counts and calculation of the calibration curve and correlation coefficient (r) by linear regression of the instrument responses for the reference standards. The statistical calculations throughout this report were performed using an automated computer spreadsheet (Microsoft Excel®) and were rounded for presentation purposes. Slight differences may be noted in hand calculations using the recoveries presented in the tables. These are due to rounding and have no effect on the scientific conclusions presented in this report. The detailed analytical data may be consulted for confirmation of the calculated results.

Several measures were taken to ensure the quality of the study results. The quality assurance unit at BASF inspected the analytical procedures for compliance with Good Laboratory Practices that included adherence to the protocol. The dates inspected are detailed in the quality

assurance unit statement. Study samples and test and reference items were maintained in secured (i.e. pad-locked) storage with limited access. Freezer temperatures were continuously monitored by electronic means.

6 SUMMARY OF METHOD

Summaries of the method parameters and characteristics are provided in Appendix B.

7 INDEPENDENT LABORATORY VALIDATION

This independent laboratory validation was successfully completed on the first trial at EPL Bio Analytical Services (reference 2). Recovery results and statistical data demonstrate BASF Analytical Method D1706/01 can be performed successfully for quantitation of BAS 510 F in Goose River and Golden Lake sediment.

EPL provided the following comments on the method:

> The method is well-written and contains enough guidance for the analyst to follow through the procedure for the first time. One method recommendation was noted upon completion of the ILV:
>
> In section 3.3 Weighing and Fortification, for treated, control, and fortified samples, it is instructed to weigh 5 g of sediment. The weight range was not defined. Since in section 4.2.3 Calculation of Residues and Recoveries, the method suggests that for the procedural recoveries the sample weight will be considered 5 g in the final calculation of residues [mg/kg], and the sample weight to be 5 ± 0.1 g for fortification samples, it is recommended to clarify that the sample weight range for all samples is 5 ± 0.1 g.

Table 2. Summary Parameters for the Analytical Method Used for the Quantitation of Residues of BAS 510 F in sediment

Method ID	BASF Analytical Method No. D1706/01
Analyte(s)	Residues of BAS 510 F in sediment
Extraction solvent/technique	Samples are extracted by shaking with methanol/sodium acetate buffer.
Cleanup strategies	Samples are diluted with water and mixed prior to analysis.
Instrument/Detector	Liquid chromatography (LC) column with detection by positive ion electrospray ionization tandem mass spectrometry (ESI-MS/MS) monitoring the following ion transitions: : m/z 343→307and 343→271for BAS 510 F All analyses are performed using a Waters Aquity UPLC system equipped with an XBridge BEH C18 column (2.1 x 50 mm, 1.7µm particle size) using a mobile phase gradient of water:methanol, each acidified with 0.1% formic acid (flow rate 600 uL/minute). Detection is obtained with a Sciex API 6500 Mass Spectrometer.
Standardization method	Direct comparison of the sample peak responses to those of external standards
Stability of std solutions	Stock and fortification solutions of BAS 510 F are prepared in methanol, and calibration standards are prepared by serial dilution of the intermediate standards using methanol/9.8mM sodium acetate(aq)/10.2mM acetic acid(aq)/water (8/1/1/90 v/v/v/v). BAS 510 F was shown to be stable in calibration standard solutions prepared by serial dilution of the intermediate standards with methanol/9.8mM sodium acetate(aq)/10.2mM acetic acid(aq)/water (8/1/1/90 v/v/v/v) and held under refrigeration for at least 30 days. Stability of BAS 510 F in methanol for stock/fortification solutions was determined in another study (Reference 1). A summary of the stability of the analyte in standard solutions is provided in Appendix E. During the course of this study, all solutions were held under refrigeration and all solutions were used within the demonstrated time period of stability.
Retention times	See Appendix B. for typical retention times

Table 3. Characteristics for the Analytical Method Used for the Quantitation of Residues of BAS 510 F in Sediment Matrices

Analyte	Residues of BAS 510 F in sediment
Equipment ID	Waters Aquity UPLC system equipped with an Acquity BEH C18 column (2.1 x 50 mm, 1.7µm particle size) using a mobile phase gradient of water:methanol, each acidified with 0.1% formic acid (flow rate 600 uL/minute). Detection is accomplished with a Sciex API 6500 Mass Spectrometer.
Limit of quantitation (LOQ)	The validated LOQ for residues in sediment is 0.005 mg/kg for BAS 510 F, which corresponds to a concentration in the final volume of 0.05 ng/mL for BAS 510 F.
Limit of detection (LOD)	The limit of detection was set at 20% of the LOQ, equivalent to 0.001 mg/kg for BAS 510 F. The lowest standard for each analyte in the calibration curve has good detectability (signal to noise ratio greater than 3:1).
Accuracy/Precision	The range of percent recoveries and coefficient of variation for each analyte, sediment type, transition (primary and secondary), and chromatographic method tested indicate acceptable accuracy/precision (overall mean within 70 to 120%, RSD ≤ 20%) in the range of spiking levels (0.005 & 0.05 mg/kg).
Reliability of the Method/ [ILV]	A successful independent laboratory validation (ILV) has been conducted for BASF Analytical Method No. D1706/01 for the determination of residues of BAS 510 F in sediment. The values obtained are indicative of the reliability of Method No. D1706/01.
Linearity	The method-detector response, for the method validation sets, was linear over the 0.01 to 0.5 ng/mL range for parent ($r \geq 0.9991$ for all analytes).
Specificity/ Selectivity	The control chromatograms generally have no peaks above the limit of detection and the spiked sample chromatograms contain only the analyte peak of interest. The level detected is below the method limit of detection (<20% of the LOQ) and does not interfere with the quantification of BAS 510 F in sediment. Peaks were well-defined and symmetrical. There appeared to be no carryover to the following chromatograms.
Matrix effects	An evaluation of matrix effects showed that the matrix load in the samples from each sediment type had no significant influence on analysis (matrix effects <20%); therefore, the validation samples were analyzed primarily using solvent-based calibration standard solutions.
Confirmatory technique	Two mass transitions are available for BAS 510 F. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique is not necessary for these analytes.
Time required	A set of 13 samples (one reagent blank, two controls, and 10 fortified samples for recovery experiments) requires about 8 hours of work (calculation of the results included).

1 INTRODUCTION

BAS 510 F is fungicides used against several diseases in various crops. The analytical method D1706/01 offers the possibility to determine BAS 510 F residues in sediment.

This method was developed at BASF Crop Protection in Research Triangle Park, NC.

2 MATERIALS

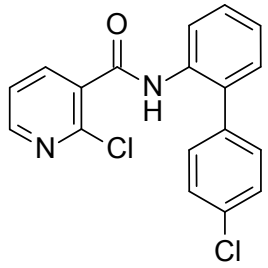
2.1 Safety

The test and reference items, as well as the chemicals required for this analysis, should be handled in accordance with good industrial hygiene and safety practice. Avoid contact with the skin, eyes and clothing. Wearing of closed work clothing is recommended. Remove contaminated clothing. Store work clothing separately. Keep away from food, drink and animal feed stuffs. No eating, drinking, smoking or tobacco use at the place of work. Hands and/or face should be washed before breaks and at the end of the shift. Details are given in the Safety Data Sheets (SDS) of the individual substances. All procedures involving organic solvents should be performed in a well-ventilated hood.

Disposal of samples and chemicals must be done in compliance with on-site safety policies and procedures.

2.2 Test and Reference Items

Test and reference items should be stored according to the information provided in the certificate of analysis.

Internal-Code	BAS 510 F	
Common Name	Boscalid	
IUPAC Name	2-chloro-*N*-(4'-chlorobiphenyl-2-yl)nicotinamide	
BASF Reg. No.	300355	
CAS-No.	188425-85-6	
Molecular Formula	$C_{18}H_{12}Cl_2N_2O$	
Molecular Weight	343.2	

2.3 Equipment

Equipment	Size, Description	Manufacturer	Catalog No.
Balance, Analytical	Model AT100	Mettler	----
Beakers	Various Sizes	PYREX Brand, VWR Scientific Products	13922-029
Centrifuge	Allegra 6R	Beckman Coulter	----
Culture tube caps	16 mm	VWR	60828-768
Culture Tubes	Glass,disposable, 16x100mm size	Fisher	14-961-29
Cylinder, Graduated	Various sizes	Various	----
HPLC Column : BEH C18	50 x 2.1 mm, 1.7 µm particle size	Waters	186002350
LC	Acquity UPLC	Waters	---
LC Vials	2 mL injection vials	National Scientific	C400-79
Mechanical shaker	KS501 digital	IKA Labortechnik	---
MicroMan pipettes	10-1000 µL	Gilson	M-25, M-50, M-250, M-1000
MS/MS	API 6500 series	AB Sciex	---
Ultrasonic Bath	Model FS 7652H	Fisher Scientific	---
Various Flask, Volumetric	100, 50, 25 ,10 and 5 mL	Various	---
Vial, Filter	PTFE eXtreme FV 0.45 µm	Thomson Filter Vials	85540-200
Volumetric pipettes	Various sizes	VWR	---
Vortex mixer	Genie 2	VWR	58816-121
French Square Jars	250mL	VWR	----

Note: The equipment and instrumentation listed above may be substituted by that of similar specifications. The applicability is confirmed if the recoveries of the fortification experiments are in the expected concentration range.

2.4 Reagents

2.4.1 Chemicals

Chemical	Grade	Manufacturer/Supplier	Catalog No.
Formic acid	>95%	Sigma-Aldrich	F0507-4X100mL
Methanol	HPLC Grade	EMD	MX0475P-1
Water, e.g. Baker® or Millipore®	Gradient Grade	BDH ARISTAR PLUS	87003-652
Sodium acetate, anhydrous	ACS Grade	Amresco	0602-500G
Acetic Acid, glacial	ACS Grade	Amresco	0714-500ML

Note: Equivalent reagents and chemicals from other suppliers may be substituted.

2.4.2 Solutions and Solvent Mixtures

Description	Code	Composition
0.2 M Sodium acetate (aq)	**S1**	0.2 M Sodium acetate (aq) Add 16.4 g of sodium acetate (anh) and 1 L of H_2O into a, e.g., 1 L Erlenmeyer flask and mix well to ensure complete homogeneous solution.
0.2 M Acetic acid (aq)	**S2**	0.2 M Acetic acid (aq) Add 11.5 mL of glacial acetic acid to 1 L of H_2O in a, e.g., 1 L Erlenmeyer flask and mix well to ensure complete homogeneous solution.
Sodium acetate buffer (pH = 4.6)	**S3**	0.098 M sodium acetate (aq)/0.102 M acetic acid (aq), 49/51 v/v Add 490 mL of 0.2 M sodium acetate (**S1**) and 510 mL of 0.2 M acetic acid (**S2**) into a, e.g., 1 L Erlenmeyer flask and mix well to ensure complete homogeneous solution.
Extraction solvent	**S4**	Methanol/0.098 M sodium acetate (aq)/0.102 M acetic acid (aq), 80/10/10, v/v/v Add 800 mL of methanol and 200 mL of sodium acetate buffer (**S3**) into a, e.g., 1L Erlenmeyer flask and mix well to ensure complete homogeneous solution.
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 Add 100 mL extraction solvent (**S4**) and 900 mL H_2O into a, e.g., 1L Erlenmeyer flask and mix well to ensure complete homogeneous solution.
HPLC mobile phase A	**LC1**	0.1% Formic Acid in Water Add 1000 mL of water and 1 mL of concentrated formic acid into a, e.g., 1L Erlenmeyer flask and mix well to ensure complete homogeneous solution.
HPLC mobile phase B	**LC2**	0.1% Formic Acid in Methanol Add 1000 mL of methanol and 1 mL of concentrated formic acid into a, e.g., 1L Erlenmeyer flask and mix well to ensure complete homogeneous solution.

Note: If necessary, the solutions may also be prepared in different volumes as long as the proportions are not modified.

2.4.3 Standard Solutions

Stock Solutions

Prepare a 1.0 mg/mL stock solution individually by weighing an appropriate amount of each analyte into a flask and add the required volume.

For example, to prepare 10 mL of 1.0 mg/mL stock solution of BAS 510 F in methanol, weigh 10 mg BAS 510 F into a 10 mL volumetric flask. Dissolve and dilute to mark with methanol. Ensure a complete homogeneous solution (e.g. by sonication or vortexing).

Independence of standard calibration and fortification solutions should initially be confirmed to show correct preparation of the solutions. This can be achieved for example using one of the following approaches:

- Two stock solutions are independently prepared. One is used for preparation of fortification solutions, the other for calibration standard solutions.
- Fortification and calibration standard solutions should be prepared from one stock solution in separate dilution series.

For subsequent preparations of solutions, freshly prepared solutions can be compared directly to previous standard solutions.

A correction for purity is done if the purity is ≤ 95%. If the purity is > 95 % correction is optional.

Fortification Solutions

Prepare mixed standard solutions for fortification by combining stock solutions of each analyte (see above) in a flask. Dilute volumetrically with appropriate solvents as exemplified in the table below and ensure a complete homogeneous solution (e.g. by sonication or vortexing).

Preparation of mixed Fortification solutions

Take solution (µg/mL)	Volume (mL)	Dilute with methanol to a final volume of (mL)	Concentration (µg/mL)
1000	0.5	50	10
10	5	50	1
1	5	50	0.1

Note: A different concentration scheme may be used, if other fortification levels are needed for the analysis.

If necessary, the volume of solution prepared may be changed as long as the proportions are not modified.

Calibration Standard Solutions

Prepare mixed standard calibration solutions for LC-MS/MS analysis by using the solutions that were prepared in Section "stock solutions" or "fortification solutions" in flasks. Dilute volumetrically with appropriate solvents as exemplified in the table below and ensure a complete homogeneous solution (e.g. by sonication or vortexing).

Preparation of standard solutions for calibration

Take solution (ng/mL)	Volume (mL)	Dilute with 8/1/1/90 Methanol/9.8 mM sodium acetate (aq)/10.2 mM acetic acid (aq)/H2O (FV1) to a final volume of (mL)	Concentration (ng/mL)
100	1	100	1
1	50	100	0.5
0.5	20	50	0.2
0.5	5	50	0.05
0.5	2.5	50	0.025
0.5	1	50	0.01

* in case matrix-matched standards (= instrument recovery samples) are needed for successful analysis, calibration standard solution are prepared in matrix solution, i.e., final volume of a control sample carried through the analytical procedure. Matrix-matched standards should be prepared in a way that the matrix load is at least 90% of the matrix load in the unknown samples. In addition the matrix load should be the same in all calibration standard solutions.

Note: A different concentration scheme may be used and additional standards may be prepared as needed.
If necessary, the volume of solution prepared may be changed as long as the proportions are not modified.

2.4.4 Stability of Standard Solutions

Stability of BAS 510 F in stock and fortification solutions of methanol has been demonstrated for 118 days at both room temperature and under refrigeration (Reference 1).

Stability of calibration solution will be established during the validation.

BASF recommends that stock solutions (1 mg/mL) in methanol be made fresh every 118 days. Dilution of stock solutions should be stored refrigerated no longer than one month or according to their established storage stability in a particular solvent.

3 ANALYTICAL PROCEDURE

3.1 Sample Preparation

Samples must be sufficiently homogenized beforehand to assure that the aliquot taken for residue analysis is representative for the whole sample.

3.2 Sample Storage

Samples are stored refrigerated (approx. 4 °C) prior to analysis.

3.3 Weighing and Fortification

For treated samples and control samples, weigh 5±0.1 g of sediment sample into a glass container (e.g. French jar)

For fortified samples, weigh 5±0.1 g of sediment sample into a glass container (e.g. French jar) and add fortification solutions on the matrix.

The following scheme may be 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
Treated	5 g	-	-	-

* limit of quantification

Note: Volume of spiking solution added to generate the fortified sample should not exceed 1% of sample weight or volume.

3.4 Extraction of Sample Material

Add 50 mL of extraction solvent **S4** to sample shake at 300 rpm for 60 min. Transfer a approx. 10 mL aliquot to a culture tube and centrifuge sample at ~3500 rpm for 5 min.

3.5 Preparation for Measurement

a) Transfer 0.1 mL of extract solution to a culture tube and add 0.9 mL of water. For residue values higher than the calibration curve, dilute the samples with **FV1** as needed to fit into the calibration curve.

Filter samples through a 0.45 µm PTFE filter and transfer to an LC vial for analysis.

Note: Samples may be filtered through a syringe filter or using LC filter vials. If using a syringe filter, discard the first few drops of sample before collecting the remainder of filtrate.

3.6 Influence of matrix effects on analysis

During method development it was demonstrated that the matrix load in the samples had no significant influence on the analysis (i.e., matrix effects < 20%). Therefore, samples can be analyzed using calibration standard solutions prepared in 8/1/1/90 Methanol/9.8 mM sodium acetate (aq)/10.2 mM acetic acid (aq)/H_2O (**FV1**).

3.7 Stability of Extracts and Final Volumes

Stability in extract and final volume solutions will be determined as part of method validation.

4 QUANTIFICATION AND CALCULATION

4.1 Set-up of the analytical run

A sequence for measurement generally consists of:
- o Calibration standards
- o Control samples
- o Procedural recovery samples
- o Unknown samples
- o Instrument recovery sample

Reagent Blanks or blanks can also be injected if necessary. Each injection set should begin and end with an injection of a calibration standard. Standards should be interspersed with samples. Each calibration standard should be at least injected twice. At least 5 calibration levels need to be injected.

4.2 Instrumental analysis

4.2.1 Instrumentation and Conditions

	Parameter		
Chromatographic System	Waters Acquity		
Analytical-column	Acquity BEH C18, 2.1 x 50 mm, 1.7µm		
Column Temperature	50°C		
Injection Volume	10-50 µL(depending on the sensitivity of the instrument)		
Mobile Phase A Mobile Phase B	Water / formic acid, 1000/1, v/v Methanol / formic acid, 1000/1, v/v		
Flow Rate	600 µL/min		
Gradient (including wash and equilibration)	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	Sciex API 6500 Mass Spectrometer		
Ionization	Electrospray (ESI)		
Ionization Temperature	550 °C		
Analyte	**Transitions**	**Polarity**	**Expected Retention Time**
BAS 510 F	343 --> 307* 343 --> 271	Positive	~2.0 min

* proposed as quantification transition. Any of these transitions could be used for quantitation in case interference is observed at the same retention time

Note: Instruments with similar specifications may substitute the equipment listed above. The instruments used are applicable for analysis if the recoveries of the fortification experiments are in the acceptable range.
In general a divert valve is used to reduce the matrix load on the detection system.
Instrument conditions, e.g. injection volumes, columns, gradient steps or mass transitions may be modified, but any changes must be recorded in the raw data. Changes are acceptable, when the recoveries of the fortification experiments are in the acceptable range.
Other parameters like gas flows and voltages are depended of the equipment used and therefore not listed. Those parameters may need to be adapted for the instrument being used.

4.2.2 Calibration procedures

Calculation of results is based on peak area measurements using a calibration curve. At least 5 calibration levels need to be injected (e.g., required for enforcement). The calibration curve is obtained by direct injection of BAS 510 F standards for LC-MS/MS, and it is recommended to use a range of 0.5 ng/mL to 0.01 ng/mL. Other concentrations of high standards may be used for calibration provided instrument response is linear over the range. In a given injection run, the same injection volume is used for all samples and standards.

Linear calibration functions are preferred for evaluation. If other functions are used (e.g. quadratic), this should be fully justified.

4.2.3 Calculation of Residues and Recoveries

Calculation of results is based on area measurements.
For the procedural recoveries, the sample weight will be considered 5 g in the final calculation of residues [mg/kg]. The method requires that the sample weight to be 5 ± 0.1 g for fortification samples. The recovery is the percentage of the fortified amount (µg or ng), which is recovered through the method and the weights cancels out, as shown in the equation below, during the final calculation step.

The residues of BAS 510 F in mg/kg are calculated as shown in equations **I** and **II**:

I. **Concentration [ng/mL]** $= \frac{\text{Response} - \mathit{Intercept}}{\mathit{Slope}} = C_A$

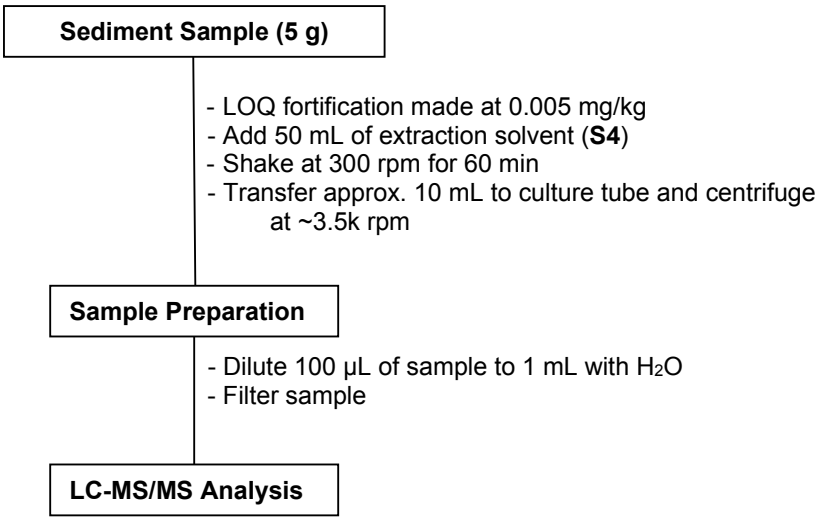
II. **Residue [mg/kg]** $= \frac{V_{end} \times C_A}{G \times A_F \times 1000}$

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	=	Aliquotation factor
1000	=	Factor remaining after all unit conversions

The recoveries of spiked compounds are calculated according to equation **III**:

III. **Recovery %** $= \frac{(\text{Residue in fortified sample} - \text{Residue in control}) \times 100}{\text{Amount of analyte fortified}}$

5 FLOWCHART



6 METHOD MANAGEMENT AND TIME REQUIREMENTS

The analysis of one series of samples (= 13 unknown samples, 2 fortified samples for recovery experiments, 1 blank sample) requires 1 working days (8 hours) per laboratory assistant. This time includes the calculation of the results, the preparation of the equipment as well as the reporting of all raw data under GLP.

7 CONCLUSION AND METHOD CAPABILITIES

Recoveries, Chromatograms, and Calibration Curves
Recovery data will be provided in the validation part of the analytical method D1706/01.

Limit of Quantification (LOQ) and Limit of Detection (LOD)
The limit of quantification is defined as the lowest fortification level successfully tested. The limit of quantification is 0.005 mg/kg for all analytes. The limit of detection was estimated at 20% of the limit of quantification, equivalent to 0.001 mg/kg for all analytes. The lowest standard for each analyte in the calibration curve has good detectability (signal to noise ratio greater than 3:1).

Selectivity
The tested untreated sediment samples showed no significant interferences (< 20 or 30 %) at the retention time of the analytes.

Confirmatory Techniques
The LC-MS/MS final determination for BAS 510 F is a highly selective detection technique. The quantitation is possible at two different transitions. Therefore, no additional confirmatory technique is required.

8 REFERENCES

[1] Funk, H; Mackenroth, C. Determination of the Stability of 205259 (BAS 480 F), 242009 (BAS 490 F), 285028 (BAS 505 F), and 300355 (BAS 510 F) in different solvents. Study number 41841. BASF DocID: 2000/1014856.