VALIDATION OF BASF METHOD No. D0004/1:

I. INTRODUCTION AND SUMMARY

A. PURPOSE OF STUDY

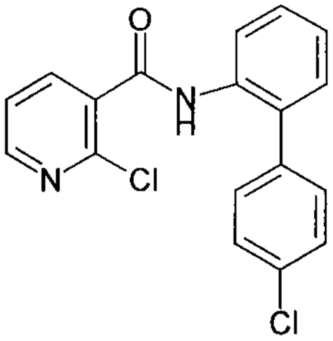
This study was conducted to validate BASF Analytical Method D0004/1. Recovery ranges and standard deviations were determined from fortified control soil samples. Recoveries of BAS 510 F and its degradates, 2-chloronicotinic acid (CNA) and 2-hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide (M510F49) were determined in four soil types. The method No. D0004/1 allows the determination of BAS 510 F and its degradates with the required limit of quantitation (0.01 ppm) in soil.

II. MATERIALS/METHODS

A. TEST AND REFERENCE SUBSTANCES

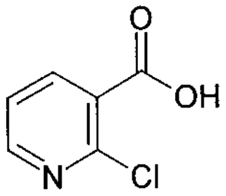
Fortification Compounds

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:	

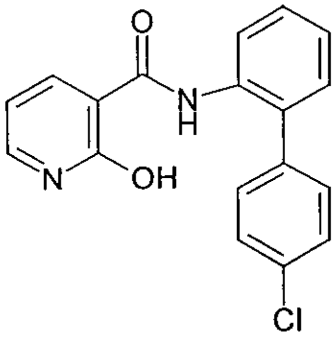


II. MATERIALS/METHODS (Continued)

Chemical Name:	2-Chloronicotinic acid
BASF Registry Number:	107371
Molecular Formula:	$C_6H_4\,ClNO_2$
Molecular Weight:	157.56
Lot No.:	01174-232
Purity:	99.8%
Structural Formula:	



BASF Registry Number:	391572
Chemical Name:	2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide
Molecular Formula:	$C_{18}H_{13}\,ClN_2O_2$
Molecular Weight:	324.77
Lot No.:	01196-217
Purity:	98.9%
Structural Formula:	



Reference Standards (used for calibration):

Same as fortification compounds.

II. MATERIALS/METHODS (Continued)

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 fortifications were made fresh every month. During the course of this study, the stability of fortification andLC-MS/MS standard solutions was examined. Solutions were stored in a refrigerator at 4°C. The following table shows the stability of the analytes in various solvent system used within the method.

SOLUTION	STABILITY (DAYS)
Stock solution BAS 510 F, BAS 510 F, 2-chloronicotinic acid, and 2-hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide (M510F49) in methanol	85
Fortification solutions in methanol for all analytes	30
LC-MS injection standards in solvent III[1]	30
BAS 510 F, 2-chloronicotinic acid, and 2-hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide (M510F49) in extraction solvent [2] with soil matrix	21
BAS 510 F, 2-chloronicotinic acid, and 2-hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide (M510F49) in LC-MS injection standards (Solvent III)[1] with soil matrix	21

[1] Solvent III: Methanol - Solvent II (50: 50, v/v)
Solvent II: water with 0.1 % formic acid and 4 mM ammonium formate

[2] Extraction Solvent Mixture:
First Extraction: Methanol
Second Extraction: Water-Methanol, 50:50, and v/v

B. TEST SYSTEM

The test system consisted of untreated soil samples obtained from trial sites of soil dissipation studies (BASF Studies 47616 and 98045) conducted in the US and Canada to validate method D0004/1.

Different soil types and depths (0-3 and 36-42 inches; German soil 2.2) were used to validate this method.

Soil samples were obtained from Georgia, California, (Study No. 47616) and Alberta (Study No. 98045) sites and were identified as BASF Residue Control Number (RCN) 99509, 99510 and 98240, respectively. Soil characterization data for soil samples used in this study are summarized in **Table V**. The detailed soil characterization data is provided in **Appendix G**.

C. SAMPLE STORAGE AND HANDLING

The soil samples were homogenized to a consistency suitable for analysis. Bulk soil samples received from the field are homogenized using a blender or mill. Homogenized soil samples are stored frozen (<-5°C) before analysis.

D. EXPERIMENTAL DESIGN

To determine recoveries of BAS 510 F and its degradates, 2-chloronicotinic acid (CNA) and 2-hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide (M510F49), control soil samples were fortified by applying standard solutions directly to the soil prior to extraction. Samples were fortified with 0.01, 0.1 and 1.0 ppm each of BAS 510 F, 2-chloronicotinic acid (CNA) and 2-hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide (M510F49) and subsequently analyzed with the method. At least one set consisting of three fortification levels was analyzed for each soil type. Analyses of six sample sets were conducted.

E. METHOD OF ANALYSIS

BASF Analytical Method D0004/1 was developed to determine the residues of BAS 510 F and its degradates, 2-chloronicotinic acid (CNA) and 2-hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide (M510F49) in soil matrices using LC-MS/MS. The method was designed to determine the residues as individual analytes and was used for the residue analysis of soil samples collected for soil dissipation studies.

The technical procedure of this method is attached to this report as **Appendix B**. Due to different soil types and instrumentation, some other modifications were made to this validated method during residue analysis [Study protocol D58647 (**Reference 1**)] and independent laboratory validation (**Reference 2**). These modifications are provided in **Appendix C**. A brief description of these methods is provided below:

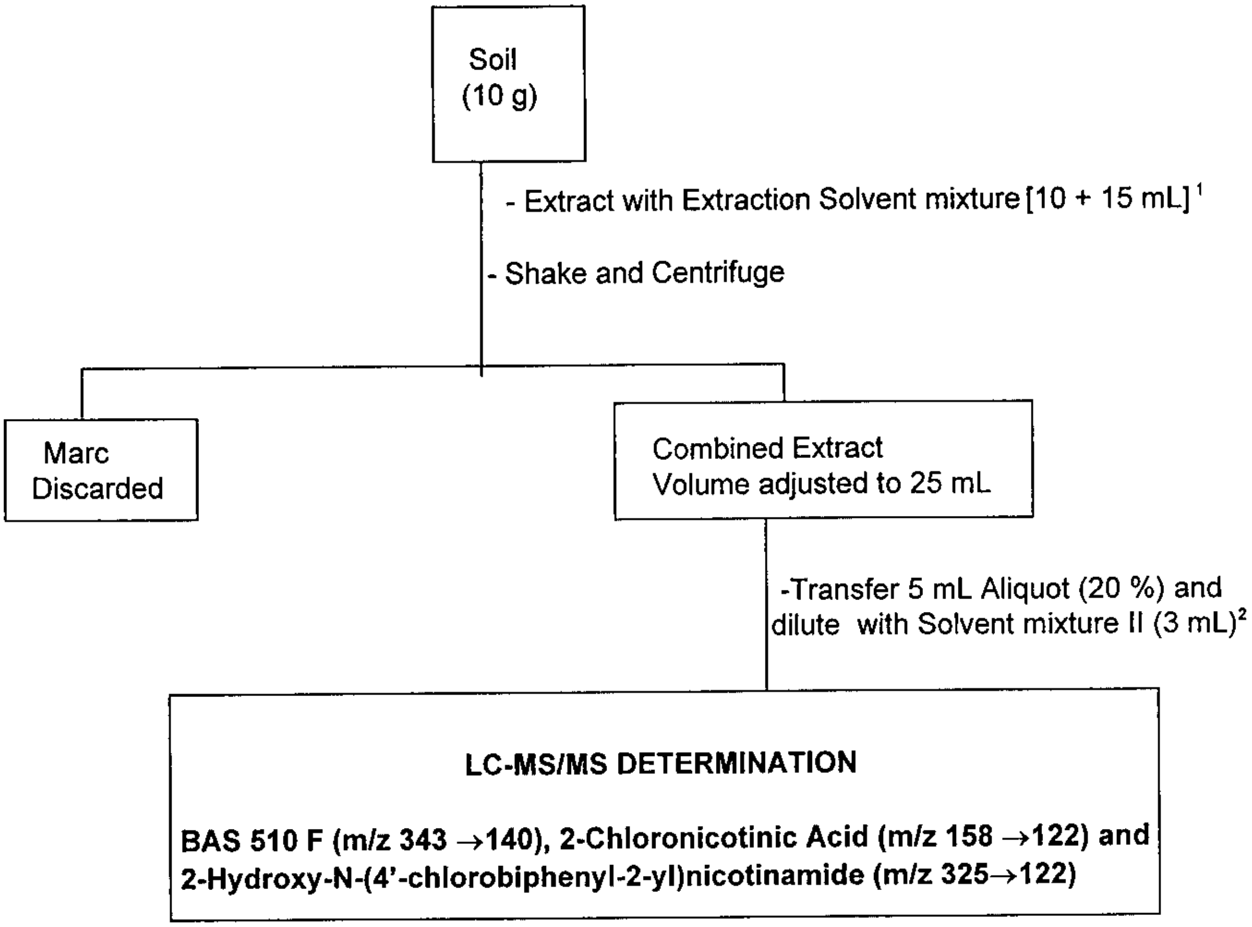
BAS 510 F and its degradates, 2-chloronicotinic acid (CNA) and 2-hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide (M510F49), were extracted from soil (10 g) by shaking with methanol followed by methanol-water (50:50, v/v).

An aliquot (20 %) of the extract is diluted with water containing 0.1 % formic acid and 4 mM ammonium formate for HPLC-MS/MS determination. The LC-MS/MS quantitation was based on following transitions: m/z 343→140, m/z 158→122 and m/z 325→122 for BAS 510 F, 2-chloronicotinic acid and 2-hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide, respectively.

A flow diagram of the analytical procedure is provided in **Figure 1**.

Specific chromatographic conditions are listed with each analysis set. Typical chromatographic parameters are provided in the technical procedure **(Section 3.5; Appendix B)**.

Figure 1. Flow Diagram for Analytical Method No. D0004/1 in Soil (BAS 510 F, 2-Chloronicotinic acid and 2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide)



[1] Extraction Solvent Mixture:
First Extraction: Methanol
Second Extraction: Water-Methanol, 50:50, and v/v

[2] Solvent Mixture II: Water with 0.1 % formic acid and 4mM ammonium formate

Figure 2. Typical Recovery Calculation for BASF Method D0004/1

Sample Number 46625/1444-35-18: Control Soils Fortified with 0.01 ppm of BAS 510 F, 2-Chloronicotinic acid and 2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide. Calculations are shown only for BAS 510 F.

Following equations were used to calculate procedural recoveries (%):

ng found per injection = Amount of analyte calculated from calibration curve

Standard curve: $$\text{ng} = \frac{\text{Peak Area} - \text{intercept}}{\text{slope}}$$

Curve Statistics: Peak area = m* Concentration + b

$$\text{mg injected} = \frac{\text{Sample weight (g) extracted}}{\text{Fv}} \times \mu\text{L injected} \times \text{F1} \times \text{F2}$$

Fv = Final volume (mL) of the extract in the extraction solvent mixture
(Section 3.2.4 of the method D0004/1)

F1 (First dilution factor) = $\frac{\text{Aliquot (mL) taken from final extract}}{\text{Dilution volume (mL)}}$
(Section 3.3.1 of the method D0004/1)

F2 (Second dilution factor): Equals 1, 0.1 and 0.01 for 0.01, 0.1 and 1.0 ppm fortification samples , respectively.

$$\text{Percent recovery (\%)} = \frac{\text{Residue (ppm) for fortified sample - Residue (ppm) for control sample}}{\text{Amount (ppm) fortified}} \times 100$$

$$\text{Residue (ppm)} = \frac{\text{ng value calculated from standard curve}}{\text{mg sample injected}} \times \text{Molecular weight conversion factor}$$

Use full computer/calculator precision in any intermediate calculations. Round only the final value.

Slope(m): 241.209 Intercept (b): -530.508

$$\text{ng (BAS 510 F) calculated from curve} = \frac{\text{Peak Area} - (-530.508)}{241.209} = \frac{12403 - (-530.508)}{241.209}$$

$$= 53.617 \text{ pg} = 0.053617 \text{ ng}$$

Figure 2 (Cont.). Typical Recovery Calculation for BASF Method D0004/1

$$\text{mg sample injected} = \frac{10.0\ (\text{g}) \times 20\ (\mu\text{L}) \times 0.625\ (\text{F1}) \times 1.0\ (\text{F2})}{25\ (\text{mL})} = 5.0\ \text{mg}$$

[molecular weight conversion factor = 1]

$$\text{Wet Sample Residue (ppm)} = \frac{0.053617}{5.0} = 0.01072$$

Wet Sample Residue (ppm) for unfortified control = 0 ppm

$$\text{Percent Recovery} = \frac{0.01072 - 0\ (\text{ppm})}{0.01\ (\text{ppm})} \times 100 = 107\%$$

[2] Residues and percent recoveries of 2-chloronicotinic acid and 2-hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide were calculated in similar fashion.

1. INTRODUCTION

1.1 Scope of the method

BAS 510 F is a new fungicide used for turf in the US and for several other crops (orchard/vineyard, and row crops) in the US, Canada and Europe. For registration of the fungicide and for establishing the DT50/90 values from field dissipation studies in these use patterns, a residue analytical method with a limit of quantitation of 0.01 mg/kg for the active ingredient and its metabolites in soil was developed. Method No. D0004/1 allows the determination of BAS 510 F and its metabolites with the required limit of quantitation in soil.

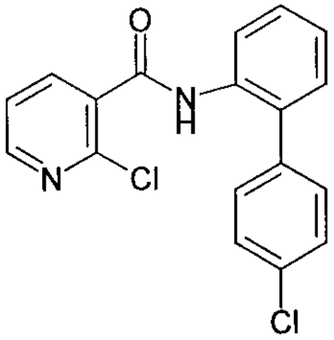
2 Materials

Standard substances are stored in a freezer (<-5°C) until use. Information on the characterization of these substances is available from BASF and is located at the Landwirtschaftliche Versuchsstation der BASF, Limburgerhof, Germany.

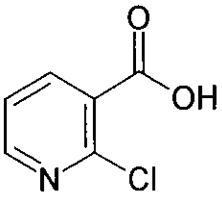
2.1 Test and Reference Substances

2.1.1 Fortification Compounds

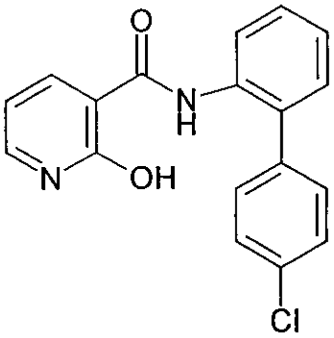
BASF Code Name:	BAS 510 F
BASF Registry Number:	300355
Chemical Name:	2-Chloro-N-(4'-chlorobiphenyl-2-yl)nicotinamide
Molecular Formula:	$C_{18}H_{12}Cl_2N_2O$
Molecular Weight:	343.21
Appearance:	White powder
Water Solubility:	4.63 mg/L
Lot No.:	01183-190
Purity:	99.3%
Structural Formula:	



Chemical Name:	2-Chloronicotinic acid
BASF Registry Number:	107371
Molecular Formula:	$C_6H_4\,ClNO_2$
Molecular Weight:	157.56
Lot No.:	01174-232
Purity:	99.8%
Structural Formula:	



BASF Registry Number:	391572
Chemical Name:	2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide
Molecular Formula:	$C_{18}H_{13}\,ClN_2O_2$
Molecular Weight:	324.77
Lot No.:	01196-217
Purity:	98.9%
Structural Formula:	



2.4.2 Reference Standards (used for calibration)

Same as fortification compounds (section 2.1.1)

2.2 Equipment -- Suggested Sizes/Suppliers, Manufacturers

Method Step	Equipment	Size, Description	Manufacturer/ Supplier	Catalog Number
2.4.2, 3.1	Balance, Analytical	Model AT100	Mettler	
Various	Balance, Top Loading	Model PM 4800	Mettler	
Various	Bar, Magnetic Stirring	2 inch lengths	Various	
3.2.2, 3.2.3	Centrifuge	Refrigerated Centrifuge Model CS-6KR	Beckmann	
3.2.1	Centrifuge Tubes Oakridge Nalgene PPCO	50 mL	VWR	21009-386
3.2.2, 3.2.3	Centrifuge Adapter	for 50 mL tubes	VWR	
Various	Cylinder, Graduated	100 mL,, 500 mL, 1000 mL	Various	
Various	Flask, Erlen Meyer, 24/40	1000 mL	Various	
Various	Flask, Volumetric	10, 25 and 50 mL	Various	
3.2.2	Funnel, long stem;	top i.d (34 mm), stem o.d.(6 mm) and stem length (52 mm)	Various	
3.3.1	Gelman PTFE acrodisc Or	0.45 um, 13 mm	Gelman Science	4422
	Millex-FH$_{13}$; Millipore (Fluoropore)	0.5 µm	Sigma	Z 22, 746-3
Various	Hot Plate, Magnetic Stirring		Various	
Various	Pipet, Volumetric	0.5, 1-10, 20, 25, 50, 100 mL	Various	
3.2.2, 3.2.3	Laboratory Shaker	Model HS501-D	Janke and Kunkel	
Various	Spatula		Various	
Various	Stopper, Teflon®	24/40	Various	
3.3	Syringes, plastic, disposable	1 mL	Various	
Various	Ultrasonic Bath	Model FS-14	Various	
3.3	Vials, HPLC Snap caps	11mm; 1.5 mL 11mm; PE w/ TFE/GR;SILICONE;	VWR Sun Brokers International	66010-539 500-350

2.2 Equipment -- Suggested Sizes/Suppliers, Manufacturers (Continued)

	Vials, Collection, PTFE screw cap	1 oz	VWR	GLC-01008
	Vials, Collection, PTFE screw cap	12 mL	VWR	66009-985
Various	Votex mixer	Genie 2	Fisher Scientific Co	12-812
3.3	LC-MS	API 3000 Biomolecular Mass Analyzer	PE Sciex	

NOTE: Other general laboratory glassware and equipment may be needed. Equipment with equivalent performance may be used, as required.

Reagents and Chemicals -- Suggested Sources

2.3.1 Chemicals

Chemical	**Grade**	**Manufacturer/ Supplier**	**Catalog Number**
Ammonium Formate	MicroSelect >99%	Fluka	09735
Formic Acid	98%	E.M. Science	FX0440-7
Glass wool	Silanized	J.T. Baker	7084-05
Methanol	High Purity	B & J	230-4
Water	High Purity	B & J	365-4

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

2.3.2 Solvent Mixtures and Their Preparation

Solvent Mixtures	Method Step
Solvent I (Extraction Solvent): Methanol-water (50:50, v/v) : Add 500 mL of methanol into a 1L graduated cylinder and dilute to the mark with water. Pour the solution to a 1L Erlenmeyer flask and mix well to ensure complete homogeneous solution.	3.2
Solvent II: Water with 0.1 % formic acid and 4 mM ammonium formate Add 1.0 mL of formic acid (98 %), about 50 –100 mL of water and 252 mg of ammonium formate into a 1L volumetric flask. Mix well to ensure complete dissolution of the ammonium formate. Dilute to the mark with water and mix well to ensure a complete homogeneous solution.	3.2.
Solvent III (Injection solvent): Methanol- **Solvent II** (50:50, v/v) : Add 500 mL of methanol into a 1L graduated cylinder and dilute to the mark with **Solvent II**. Pour the solution to a 1L Erlenmeyer flask and mix well to ensurea complete homogeneous solution.	2.4.2 , 2.4.3 and 3.3.1
LC-MS Mobile Phase A: Water with 0.1 % formic acid and 4 mM ammonium formate	3.5
LC-MS Mobile Phase B: Methanol with 0.1 % formic acid and 4 mM ammonium formate	3.5

2.4 Standard Solutions

2.4.1 Standard Solution Storage Stability

Standard solutions are kept refrigerated. The storage stability of standard solutions made in methanol and any other solvent will be established during the course of the study. BASF recommends that stock solutions (1 mg/mL in methanol) be made fresh every three months. Dilution of stock solutions should be stored refrigerated no longer than one month or according to their established storage stability in a particular solvent.

2.4.2 Standard Solutions

2.4.2.1 Stock solutions (1 mg/mL)

BAS 510 F

Prepare a 1.0 mg/mL BAS 510 F stock solution by weighing an appropriate amount of BAS 510 F into a volumetric flask. Dissolve with methanol and dilute to mark. For example, to prepare a 25 mL stock solution, place 25.0 mg of BAS 510 F into a 25 mL volumetric flask. Dissolve and dilute to mark with methanol. Sonicate and vortex to ensure a complete homogeneous solution.

2-Chloronicotinic acid

Prepare a 1.0 mg/mL stock solution by weighing an appropriate amount of 2-chloronicotinic acid into a volumetric flask. Dissolve with methanol and dilute to mark. For example, to prepare a 25 mL stock solution, place 25.0 mg of 2-chloronicotinic acid into a 25 mL volumetric flask. Dissolve and dilute to mark with methanol. Sonicate and vortex to ensure a complete homogeneous solution.

2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide

Prepare a 1.0 mg/mL stock solution by weighing an appropriate amount of 2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide into a volumetric flask. Dissolve with methanol and dilute to mark. For example, to prepare a 25 mL stock solution, place 25.0 mg of 2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide into a 25 mL volumetric flask. Dissolve and dilute to mark with methanol. Sonicate and vortex to ensure a complete homogeneous solution.

2.4.2.2 Mix Standards for Fortifications

BAS 510 F, 2-Chloronicotinic acid and 2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide

Prepare a 10 µg/mL mixed standard solution for fortification by combining 0.5 mL of each of the BAS 510 F, 2-Chloronicotinic acid and of 2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide stock solutions (2.4.2.1) into a 50 mL volumetric flask to the volumetric flask. Dilute to mark with methanol. Sonicate and vortex to ensure a complete homogeneous solution. Prepare serial dilutions of this combined solution as needed. Suggested concentrations of mixed standards for fortifications are 10 µg/mL, 1.0 µg/mL, and 0.1 µg/mL, in methanol.

2.4.2.3 Injection Standard Solutions of BAS 510 F, 2-Chloronicotinic acid and 2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide for LC-MS/MS Analysis (Calibration Standards): 25, 5.0, 2.5, and 1.25 pg/µL in Solvent mixture III

Prepare a 25.0 pg/µL mixed injection standard solution by transferring an appropriate amount of the 1.0 µg/mL of each fortification solutions (2.4.2.2) with a volumetric pipet into a volumetric flask containing **Solvent mixture III.** Typically add 2.5 mL of the 1.0 µg/mL of each fortification solutions into a 50mL volumetric flask and dilute to the mark with **Solvent mixture III**. Prepare serial dilutions of this solution as needed. Suggested concentrations of mixed standards are 25, 5.0, 2.5 and 1.25 pg/µL, in **Solvent mixture III.**

NOTE: Use amber bottles with Teflon®-lined screw caps as storage containers for standard solutions. Suggested standard concentrations are listed here. A different concentration scheme may be used and additional standards may be prepared as needed.

3. Analytical Procedure

3.1 Sample Preparation

Bulk soil samples received from the field are homogenized using a blender or mill. Homogenized soil samples are stored frozen (<-5°C) before analysis. Weigh a 10 g (or to the nearest tenth of a gram) aliquot of the soil sample into a 50 mL centrifuge bottle.

3.2 Fortification and Extraction

3.2.1 For the fortification samples, add an appropriate volume of a mixed standard solution of BAS 510 F, 2-Chloronicotinic acid and 2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide to the respective control sample by volumetric pipet. For example, for a 0.01 ppm fortification sample, pipet 1 mL of the 0.1 µg/mL mixed standard solution of BAS 510 F, 2-Chloronicotinic acid and of 2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide onto a control sample.

3.2.2 Add 10 mL methanol into the centrifuge bottle containing the soil and shake at 300 RPM for 30 minutes. Centrifuge at 3000 rpm for 5 minutes at 20° C. Attach a funnel plugged with glass wool into a 25 mL volumetric flask, transfer the supernatant by decantation through the funnel and collect.

3.2.3 Add 15 mL aliquot of **Solvent mixture I** into the soil marc, sonicate and vortex to loosen the soil and allow to mix to consistency. Use a flat head spatula to break the soil marc and mix well to obtain a homogeneous suspension. Repeat the extraction step above (3.2.2) for 30 minutes. Centrifuge at 3000 rpm for 5 minutes and transfer the supernatant into the above 25 mL volumetric flask by decantation through the funnel.

NOTE: Centrifugation must be continued until the solid residue forms a compact pellet.

3.2.4 Bring the volume of the combined extracts to 25 mL with the methanol. **Mix well to obtain a homogeneous extract.**

NOTE: Extract should be stored at the refrigerator

3.3 Sample Preparation for LC-MS/MS Analysis

3.3.1 For LC-MS/MS determination, transfer a 5 mL aliquot of the extract (3.2.4) into a vial (~ 10 mL capacity) and add 3 mL of **Solvent mixture II**. Sonicate and vortex to ensure a complete homogeneous solution.

Typically the following procedures are used to prepare the samples for analysis:

3.3.2 **For control and 0.01 ppm fortifications**, filter the solution through a syringe filter (a 0.5 micron Fluoropore disc fitted to 1.0 mL disposable plastic syringe). Transfer the sample solution (3.3.1) with a glass disposable pipette to the syringe, discard the initial 100- 200 µL of the filtrate and collect the filtrate (about 1-2mL) into an injection vial.

3.3.3 **For 0.1 ppm fortifications**, take 1 mL of the sample solution (3.3.2) and dilute to 10 mL with **solvent mixture III**. Sonicate and vortex to ensure a homogeneous solution. Filter the solution into the injection vial using the procedure above.

For 1.0 ppm fortifications, take 1 mL of the sample solution (3.3.1) and dilute to 100 mL with **solvent mixture III**. Sonicate and vortex to ensure a homogeneous solution. Filter the solution into the injection vial using the procedure above.

The sample is ready for injection.

A flow chart of the analytical procedure is presented in **Figure 1**.

3.4 Moisture Determination

Results of soil analysis are reported on a "dry weight" basis for residue determination. Therefore soil sample weights must be corrected for moisture content by any method the laboratory customarily uses. But the procedural recoveries will not be corrected for moisture content of the sample. An example of a moisture determination procedure is provided in the validation report.

3.5. Instrumentation

Suggested LC-MS/MS Operating Conditions:

Instrument:	PE Sciex API 3000 Biomolecular Mass Analyzer
Inlet [HPLC System]:	PE Series 200 Micro Pump system with Series 200 Autosampler
Data System:	MassCrom 1.1
Column:	Inertsil ODS-3 5μ, 100 X 2.1 mm, [Metachem ; Serial No. 9K150217]
Injection:	Typically 20 μL
Mobile Phase: [Gradient]	A = water with 4 mM ammonium formate and 0.1% formic acid B = methanol with 4 mM ammonium formate and 0.1% formic acid **Time (min.)** **Composition** 0.0 80% A + 20% B 0.1 10% A + 90% B 0.6 10% A + 90% B 2.6 80% A + 20% B 2.7 80% A + 20% B Run every 5 minutes
Flow Rate:	400 μL/minute

	BAS 510 F	**2-Chloronicotinic acid**	**2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide**
Expected Retention Times	2:02 minutes	1.41 minutes	2.09 minutes
Transitions:	343→140	158→122	325→122
Ionization Mode:	Positive ion for all analytes; APCI with heated nebulizer (400°C)		

NOTES:

1. **The equipment listed was used for method development and validation. Other equivalent hardware may be used. The use of a guard column is optional.**
2. **The recommended instrument parameters were found to be optimal for the instrument used for the method validation. The exact values used must be optimized for each instrument.**

3. **The recommended chromatographic systems were found to be optimal for the types of instrument used for the method validation. Different chromatographic systems might be necessary for different types of instrument.**

3.6 Calibration Procedures

Calculation of results is based on peak area measurements using a calibration curve. The standard curve is obtained by direct injection of 20 µL of the mixed BAS 510 F, 2-Chloronicotinic acid, and 2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide standards for LC-MS/MS in the range of 0.5 pg/µL to 5.0 pg/µL. In a given injection run, the same volume is used for all samples and standards. Typical standard amounts injected on-column range as follows: 12.5, 25.0, 50.0 and 100 pg.

Prepare calibration curves by plotting the peak area (monitoring transitions 343→140, 158→122, and 325→122 for mixed BAS 510 F, 2-Chloronicotinic acid, and 2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide, respectively, versus the weight of mixed BAS 510 F, 2-Chloronicotinic acid, and 2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide, respectively, using a linear least squares working curve in the form $y = bx + c$.

Establish the stability of the detection response by injecting several concentrations of standards. For analysis, alternate samples and standards. For each injection set, the set should begin and end with standard injections, and each standard level should be injected at least in duplicate.

Note: It is advisable to "stabilize" on column retention time of the analytes before injecting the first sample of an analytical series.

3.7 Limit of Quantitation and Limit of Detection

The limit of quantitation is defined as the lowest fortification level successfully tested. The limit of quantitation is 0.01 ppm for BAS 510 F and its metabolites, 2-Chloronicotinic acid, and 2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide. The limit of detection has not been determined, but the lowest standard for each analyte in calibration curve has good detectability (signal to noise ratio greater than 3:1).

4. Calculation of Results

Calculation of results is based on peak area measurements. A typical calculation to determine the residues of BAS 510 F and its metabolites are shown in page 19 of this report.

5. Time Requirement for Analysis

The time required for a set of 8 samples (either 6 fortified and 2 controls or 5 treated samples, 2 fortified and 1 control) is approximately 6 person-hours, or 1 calendar day, provided that no special problems arise, such as matrix interference.

6. Confirmatory techniques

The method allows for the determination of BAS 510 F and its metabolites using LC-MS/MS which is a highly selective and self confirmatory detection technique. Therefore, no confirmatory technique is required.

7. Potential problems

In case of clay soil, the soil marc has to be broken completely after the first centrifugation in the extraction step in order to obtain acceptable recovery of 2-chloronicotinnnic acid. The glass ware used for the method should be thoroughly rinsed with acetone to prevent contamination. 2- Chloronicotinnnic acid is relatively less sensitive as compared to the BAS 510 F and 2-hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide when LC-MS/MS detection was used for quantitation.

8. Safety and health considerations

All procedures involving organic solvents should be performed in a well-ventilated hood. Personal protective equipment (gloves, lab coats) should be worn while performing this method. Read all label statements and precautions.

This section of the report summarizes all of the changes made to the method D0004/1

Modification No. 1:

In order to expedite the residue analyses (reduce run time, high sample throughput), it was required to contract out residue analyses. A different instrument (LC-MS/MS detection; Sceix API 365, Perkin Elmer) was used to analyze the samples for residue analyses. There were also some minor changes in different sections of the method to transfer the chromatographic method from PE Sceix API 3000 to PE Sceix API 365. A flow diagram of this modified method is provided in Figure 1 of this section (**APPENDIX C**). This modification was made to achieve the required sesitivity for 2-chloronicotinic acid. Method modifications as well as instrument conditions are described below:

Section 2.4.2.3 Use water-methanol (80:20, v/v) with 0.3% formic acid and 4mM ammonium formate instead of Solvent mixture III

Section 3.2.1. Use aTeflon centrifuge bottle (50 mL capacity, VWR Catalog No. 21009-477) for extraction vessel.

Section 3.3.1: Not Used

Section 3.3.2: For LC-MS/MS determination of control and 0.01 ppm fortifications, transfer a 1 mL aliqout of the extract (Section 3.2.4) and add 0.5 mL of HPLC water with 0.3% formic acid and 4 mM ammonium formate solution to the sample. Filter an aliquot of the solution through a syringe filter as per method. Larger aliquot may be used, but keeping the same ratio of aliquot to buffer.

Alternatively, filter an aliquot of the solution through a syringe filter as per method and collect the filtrate in a 1 mL volumetric flask. Transfer the entire sample to an injection vial. Add 0.5 mL of HPLC water with 0.3% Formic Acid and 4 mM ammonium formate solution to the sample.

Section 3.3.3: For 0.1 ppm fortifications, transfer 1 mL of the sample solution from 3.3.2. and dilute to 10 mL with water-methanol 80:20, v/v with 0.3% formic acid and 4mM ammonium formate. Filter an aliquot of the solution through a syringe filter as per method.

Section 3.5: Instrument conditions as well as the parameters are described below:

<table>
<tr><td>Instrument:</td><td colspan="3">PE Sciex API 365 Biomolecular Mass Analyzer</td></tr>
<tr><td>Inlet [HPLC System]:</td><td colspan="3">HP1100 HPLC system with autosampler</td></tr>
<tr><td>Column:</td><td colspan="3">Phenomenex, Polar RP 80A, 75 x 4.6 mm; Column temperature 60 °C</td></tr>
<tr><td>Injection:</td><td colspan="3">Typically 80 µL</td></tr>
<tr><td>Mobile Phase:
[Gradient]</td><td colspan="3">A = water with 4 mM ammonium formate and 0.1% formic acid
B = methanol with 4 mM ammonium formate and 0.1% formic acid
<table>
<tr><th>Time (min.)</th><th>%Mobile Phase A</th><th>%Mobile Phase B</th></tr>
<tr><td>0.0</td><td>90</td><td>10</td></tr>
<tr><td>0.1</td><td>90</td><td>10</td></tr>
<tr><td>0.25</td><td>1</td><td>99</td></tr>
<tr><td>4.85</td><td>1</td><td>99</td></tr>
<tr><td>5.05</td><td>90</td><td>10</td></tr>
<tr><td>7.55</td><td>90</td><td>10</td></tr>
</table></td></tr>
<tr><td>Flow Rate:</td><td colspan="3">400 µL/minute</td></tr>
<tr><td></td><td>BAS 510 F</td><td>2-Chloronicotinic acid</td><td>2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide</td></tr>
<tr><td>Expected Retention Times</td><td>4.68 minutes</td><td>3.84 minutes</td><td>4.81 minutes</td></tr>
<tr><td>Transitions:</td><td>343→140</td><td>158 MS only</td><td>325→122</td></tr>
<tr><td>Ionization Mode:</td><td colspan="3">Positive ion for all analytes; APCI with heated nebulizer (400°C)</td></tr>
</table>

Modification No. 2:

This modification of the method was used during Independent Laboratory Validation. A different LC-MS/MS instrument was used for analyses. There were also some minor changes in the chromatographic method from PE Sceix API 3000 to MicroMass Quattro LC. Method modifications as well as the instrument conditions are described below .

Modification No. 2 (Continued):

LC-MS/MS System:

The following conditions, based on Section 3.5 of the supplied method, were used for LC-MS/MS analysis of BAS 510 F, 2-Chloronicotinic Acid, and 2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide.

HPLC:	Gilson 305 and 306 pumps, Gilson 805 manometric module, Gilson 811C dynamic mixer, and Gilson 235 autosampler
Analytical Column:	Metachem, Intersil ODS-3, 5 µm, 100 x 2.1 mm, part # 0396-100x021
Mobile Phase A:	Water with 0.1% formic acid and 4 mM ammonium formate
Mobile Phase B:	Methanol with 0.1% formic acid and 4 mM ammonium formate

HPLC Gradient:

Time (minutes)	Composition
0.0	20% B
0.1	50% B
1.1	50% B
2.0	70% B
3.0	90% B
4.0	90% B
4.5	20% B
4.6	20% B
7.0	20% B

Flow Rate:	400 µL/minute
Injection Volume:	50 µL
Data Acquisition:	MassLynx, v. 3.2 build 004
Retention Times:	BAS 510 F, ~3.75 minutes 2-Chloronicotinic acid, ~1.41 minutes 2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide, ~ 4.17 minutes
Mass Spectrometer:	MicroMass Quattro LC
Ionization:	Positive Ion, APCI (650°C)

Modification No. 2 (Continued):

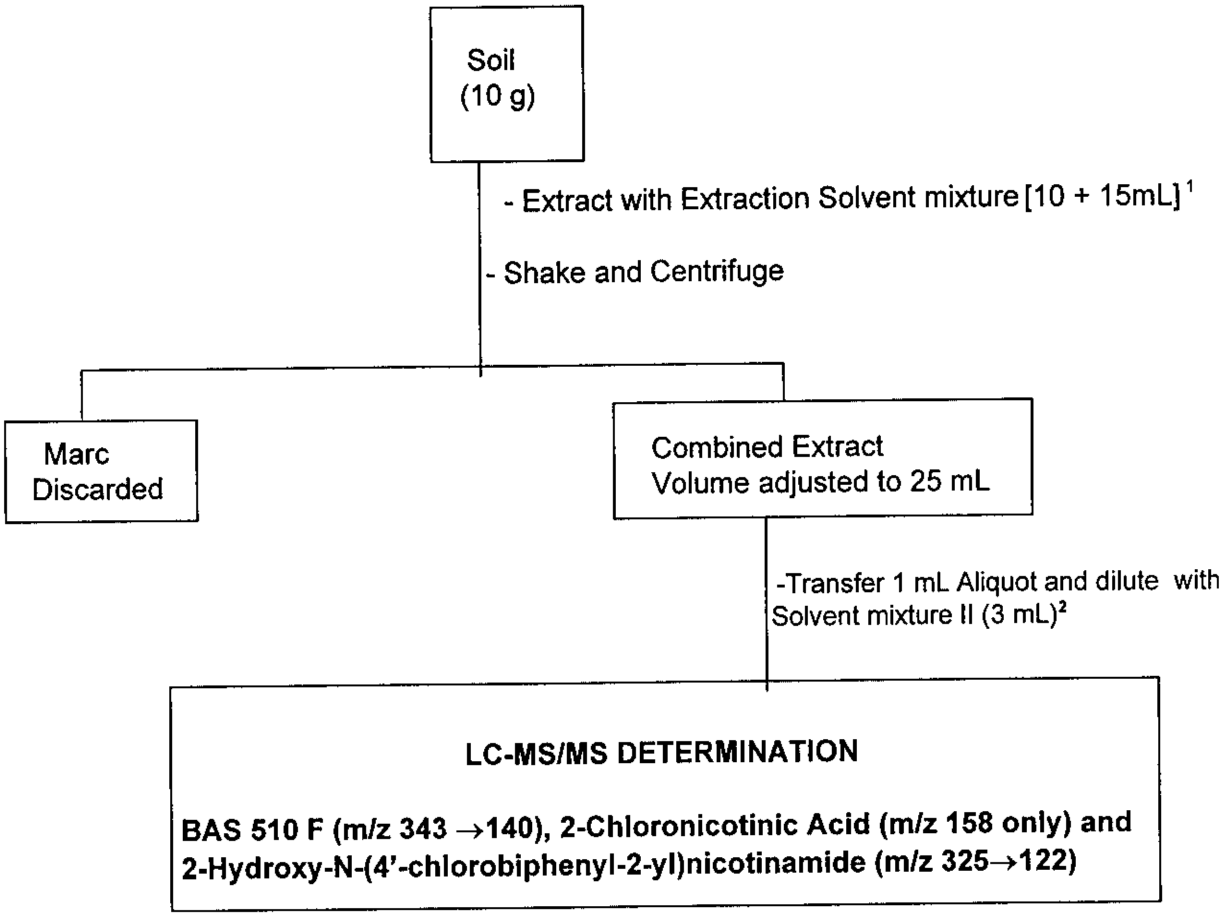
Ions Monitored: m/z 343→140 (BAS 510 F); m/z 158→122 (2-Chloronicotinic acid)

m/z 325→122 (2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide)

Summary :

In general, during the routine residue analysis, a majority of the soil samples were analyzed using Method D0004/1. If the procedural recoveries were lower than 60-65 % in a particular analysis set or adequate sensitivity in LC-MS/MS quantitation was not achieved for 2-Chloronicotinic acid, Method D0004/1 with modification described in Modification No. 2 was used for the re-extraction/reanalysis.

Figure 1. Flow Diagram for Analytical Method No. D0004/1 in Soil with modification (BAS 510 F, 2-Chloronicotinic acid and 2-Hydroxy-N-(4'-chlorobiphenyl-2-yl)nicotinamide)



[1] Extraction Solvent Mixture:
First Extraction: Methanol
Second Extraction: Water-Methanol (50:50, and v/v)

[2] Solvent Mixture II: Water with 0.1 % formic acid and 4mM ammonium formate

1. INTRODUCTION

1.1 Scope of the method

BAS 510 F is a new fungicide used for turf in the US and for several other crops (orchard/vineyard, and row crops) in the US, Canada and Europe. For registration of the fungicide and for establishing the DT50/90 values from field dissipation studies in these use patterns, a residue analytical method with a limit of quantitation of 0.01 mg/kg for the active ingredient and its metabolites in soil was developed. The method No. D0004 allows the determination of BAS 510 F and its metabolites with the required limit of quantitation in soil.

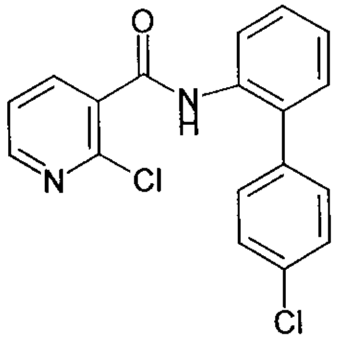
2. Materials

Standard substances are stored in a freezer (<-5°C) until use. Information on the characterization of these substances is available from BASF and is located at the Landwirtschaftliche Versuchsstation der BASF, Limburgerhof, Germany.

2.1 Test and Reference Substances

2.1.1 Fortification Compounds

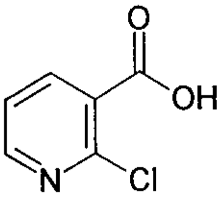
BASF Code Name:	BAS 510 F
BASF Registry Number:	300355
Chemical Name:	2-Chloro-N-(4'-chlorobiphenyl-2-yl)nicotinamide
Molecular Formula:	$C_{18}H_{12}Cl_2N_2O$
Molecular Weight:	343.21
Appearance:	White powder
Water Solubility:	4.63 mg/L
Lot No.:	01183-190
Purity:	99.3%
Structural Formula:	



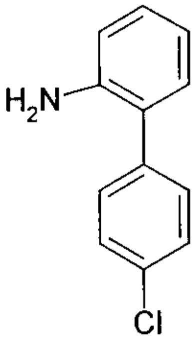
Chemical Name:	2-Chloronicotinic acid
BASF Registry Number:	107371
Molecular Formula:	$C_6H_4ClNO_2$
Molecular Weight:	157.56

2. Materials (Continued)

Lot No.: 01174-232
Purity: 99.8%
Structural Formula:



Chemical Name: 1-(4-Chlorophenyl)-2-aminobenzene
BASF Registry Number: 363487
Molecular Formula: $C_{12}H_{10}ClN$
Molecular Weight: 203.67
Lot No.: 01196-271
Purity: 98.8%
Structural Formula:



2.1.2 Reference Standards (used for calibration)

Same as fortification compounds (section 2.1.1)

2.1.3 TEST SYSTEM

The test system consisted of untreated soil samples obtained from trial sites of soil dissipation studies (BASF Studies 98016, 98017 and 98045) conducted in the US and Canada to validate method D0004. Different soil types and depths (0-3, 3-6 and 12-18 inches; German soil 2.2) were used to validate this method. Soil samples were obtained from California, (Study No. 98017), Indiana, New Jersey (Study No. 98016), and Alberta (Study No. 98045) sites and were identified as BASF Residue Control Number (RCN) 98093, 98087, 98088 and 98240, respectively. Soil characterization data for soil samples used in this study are summarized in **Table X**. The detail soil characterization data is provided in **Appendix G**.

2.2 Equipment -- Suggested Sizes/Suppliers, Manufacturers

Method Step	Equipment	Size, Description	Manufacturer/ Supplier	Catalog Number
2.4.2, 3.1	Balance, Analytical	Model AT100	Mettler	
Various	Balance, Top Loading	Model PM 4800	Mettler	
Various	Bar, Magnetic Stirring	2 inch lengths	Various	
3.2.2, 3.2.3	Centrifuge	Refrigerated Centrifuge Model CS-6KR	Beckmann	
3.2.1	Centrifuge Tubes Oakridge Nalgene PPCO	50 mL	VWR	21009-386
3.2.2, 3.2.3	Centrifuge Adapter	for 50 mL tubes	VWR	
Various	Cylinder, Graduated	100 mL,, 500 mL, 1000 mL	Various	
Various	Flask, Erlen Meyer, 24/40	1000 mL	Various	
Various	Flask, Volumetric	10, 25 and 50 mL	Various	
3.2.2	Funnel, long stem;	top i.d (34 mm), stem o.d.(6 mm) and stem length (52 mm)	Various	
3.3.1	Gelman PTFE acrodisc Or	0.45 um, 13 mm	Gelman Science	4422
	Millex-FH$_{13}$; Millipore (Fluoropore)	0.5 µm	Sigma	Z 22, 746-3
Various	Hot Plate, Magnetic Stirring		Various	
Various	Pipet, Volumetric	0.5, 1-10, 20, 25, 50, 100 mL	Various	
3.2.2, 3.2.3	Laboratory Shaker	Model HS501-D	Janke and Kunkel	
Various	Spatula		Various	
Various	Stopper, Teflon®	24/40	Various	
3.3	Syringes, plastic, disposable	1 mL	Various	
Various	Ultrasonic Bath	Model FS-14	Various	

2.2 Equipment -- Suggested Sizes/Suppliers, Manufacturers (Continued)

3.3	Vials, HPLC Snap caps	11 mm; 1.5 mL 11 mm; PE w/ TFE/GR;SILICONE;	VWR Sun Brokers International	66010-539 500-350
	Vials, Collection, PTFE screw cap	1 oz	VWR	GLC-01008
	Vials, Collection, PTFE screw cap	12 mL	VWR	66009-985
Various	Votex mixer	Genie 2	Fisher Scientific Co	12-812
3.3	LC-MS	API 3000 Biomolecular Mass Analyzer	PE Sciex	

NOTE: Other general laboratory glassware and equipment may be needed. Equipment with equivalent performance may be used, as required.

2.3 Reagents and Chemicals -- Suggested Sources

2.3.1 Chemicals

Chemical	Grade	Manufacturer/ Supplier	Catalog Number
Ammonium Formate	MicroSelect >99%	Fluka	09735
Formic Acid	98%	E.M. Science	FX0440-7
Glass wool	Silanized	J.T. Baker	7084-05
Methanol	High Purity	B & J	230-4
Water	High Purity	B & J	365-4

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

2.3.2 Solvent Mixtures and their Preparation

Solvent Mixtures	Method Step
Solvent I (Extraction Solvent): Methanol-water (50:50, v/v) : Add 500 mL of methanol into a 1L graduated cylinder and dilute to the mark with water. Pour the solution to a 1L Erlenmeyer flask and mix well to ensure complete homogeneous solution.	3.2
Solvent II: Water with 0.1 % formic acid and 4 mM ammonium formate Add 1.0 mL of formic acid (98 %), about 50 –100 mL of water and 252 mg of ammonium formate into a 1L volumetric flask. Mix well to ensure complete dissolution of the ammonium formate. Dilute to the mark with water and mix well to ensure complete homogeneous solution.	3.2.
Solvent III (Injection solvent): Methanol- **Solvent II**, 50:50, v/v : Add 500 mL of methanol into a 1L graduated cylinder and dilute to the mark with **Solvent II**. Pour the solution to a 1L Erlenmeyer flask and mix well to ensure complete homogeneous solution.	2.4.2 , 2.4.3 and 3.3.1
LC-MS Mobile Phase A: Water with 0.1 % formic acid and 4 mM ammonium formate	3.5
LC-MS Mobile Phase B: Methanol with 0.1 % formic acid and 4 mM ammonium formate	3.5

2.4 Standard Solutions

2.4.1 Standard Solution Storage Stability

Standard solutions are kept refrigerated. The storage stability of standard solutions made in methanol and any other solvent will be established during the course of the study. BASF recommends that stock solutions (1 mg/mL in methanol) be made fresh every three months. Dilution of stock solutions should be stored refrigerated no longer than one month or according to their established storage stability in a particular solvent.

2.4.2 Standard Solutions

2.4.2.1 Stock solutions (1 mg/mL)

BAS 510 F

Prepare a 1.0 mg/mL BAS 510 F stock solution by weighing an appropriate amount of BAS 510 F into a volumetric flask. Dissolve with methanol and dilute to mark. For example, to prepare a 25 mL stock solution, place 25.0 mg of BAS 510 F into a 25 mL volumetric flask. Dissolve and dilute to mark with methanol. Sonicate and vortex to ensure a complete homogeneous solution.

2.4.2 Standard Solutions (Continued)

2-Chloronicotinic acid

Prepare a 1.0 mg/mL stock solution by weighing an appropriate amount of 2-chloronicotinic acid into a volumetric flask. Dissolve with methanol and dilute to mark. For example, to prepare a 25 mL stock solution, place 25.0 mg of 2-chloronicotinic acid into a 25 mL volumetric flask. Dissolve and dilute to mark with methanol. Sonicate and vortex to ensure a complete homogeneous solution.

1-(4-Chlorophenyl)-2-aminobenzene

Prepare a 1.0 mg/mL stock solution by weighing an appropriate amount of 1-(4-Chlorophenyl)-2-aminobenzene into a volumetric flask. Dissolve with methanol and dilute to mark. For example, to prepare a 25mL stock solution, place 25.0 mg of 1-(4-Chlorophenyl)-2-aminobenzene into a 25 mL volumetric flask. Dissolve and dilute to mark with methanol. Sonicate and vortex to ensure a complete homogeneous solution.

2.4.2.2 Standards for Fortifications

BAS 510 F and 2-Chloronicotinic acid

Prepare a 10 µg/mL mixed standard solution for fortification by combining 0.5mL of each of the BAS 510 F and 2-Chloronicotinic acid stock solutions (2.4.2.1) into a 50 mL volumetric flask to the volumetric flask. Dilute to mark with methanol. Sonicate and vortex to ensure a complete homogeneous solution. Prepare serial dilution's of this combined solution as needed. Suggested concentrations of mixed standards for fortifications are 10 µg/mL, 1.0 µg/mL, and 0.1 µg/mL, in methanol.

1-(4-Chlorophenyl)-2-aminobenzene

Prepare a 10 µg/mL standard solution for fortification by combining 0.5mL of each of the 1-(4-Chlorophenyl)-2-aminobenzene stock solutions (2.4.2.1) into a 50 mL volumetric flask to the volumetric flask. Dilute to mark with methanol. Sonicate and vortex to ensure a complete homogeneous solution. Prepare serial dilution's of this solution as needed. Suggested concentrations of standards for fortifications are 10 µg/mL, 1.0 µg/mL, and 0.1 µg/mL, in methanol.

2.4.2 Standard Solutions (Continued)

2.4.2.3 Injection Standard Solutions of BAS 510 F, 2Chloronicotinic acid and 1-(4-Chlorophenyl)-2-aminobenzene for LC-MS/MS Analysis (Calibration Standards): 10, 5.0, 2.5, and 1.25 pg/µL in Solvent mixture III

Prepare a 10.0 pg/µL mixed injection standard solution by transferring an appropriate amount of the 1.0 µg/mL of each fortification solutions (2.4.2.2) with a volumetric pipet into a volumetric flask containing **Solvent mixture III** (typically add 25 mL of **Solvent mixture III** into a 50 mL volumetric flask and add 0.5 mL of the 1.0µg/mL of each fortification solutions). Dilute to the mark with **Solvent mixture III**. Prepare serial dilution's of this solution as needed. Suggested concentrations of mixed standards are 10, 5.0, 2.5 and 1.25 pg/µL, in **Solvent mixture III**.

> NOTE:
> Use amber bottles with Teflon®-lined screw caps as storage containers for standard solutions. Suggested standard concentrations are listed here. A different concentration scheme may be used and additional standards may be prepared as needed.

3. Analytical Procedure

3.1 Sample Preparation

Bulk soil samples received from the field are homogenized using a blender or mill. Homogenized soil samples are stored frozen (<-5°C) before analysis. Weigh a 10 g (or to the nearest tenth of a gram) aliquot of the soil sample into a 50 mL centrifuge bottle.

3.2 Fortification and Extraction

3.2.1 For the fortification samples, add an appropriate volume of a BAS 510 F and 2-Chloronicotinic acid standard solution to the respective control sample by volumetric pipet. For example, for a 0.01 ppm fortification sample, pipet 1 mL of the 0.1 µg/mL mixed standard solution of BAS 510 F and 2-Chloronicotinic acid onto a control sample.

3.2.2 Add 15 mL (or 10 mL in case of clay soil) methanol into the centrifuge bottle containing the soil and shake at 300 RPM for 30 minutes. Centrifuge at 3000 rpm for 5 minutes at 20 ° C. Attach a funnel plugged with glass wool into a 25 mL volumetric flask, transfer the supernatant by decantation through the funnel and collect.

2. Analytical Procedure (Continued)

3.2.3 Add 10 mL (or 20 mL in case of clay soil) aliquot of **Solvent mixture I** into the soil marc, sonicate and vortex to loosen the soil and allow to mix to consistency (see note below). Repeat the extraction step above (3.2.2) for 30 minutes. Centrifuge at 3000 rpm for 5 minutes and transfer the supernatant into the above 25 mL volumetric flask by decantation through the funnel.

NOTE: 1. In case of clay soil, use a flat head spatula to break the soil and mix well to obtain a homogeneous suspension.
2. Centrifugation must be continued until the solid residue forms a compact pellet.

3.2.4 Bring the volume of the combined extracts to 25 mL with the methanol. **Mix well to obtain a homogeneous extract.**

NOTE: Extract should be stored at the refrigerator

3.3 Sample Preparation for LC-MS/MS Analysis

3.3.1 For LC-MS/MS determination, transfer a 5 mL aliquot of the extract (3.2.4) into a vial (~ 10 mL capacity) and add 3 mL of **Solvent mixture II**. Sonicate and vortex to ensure a complete homogeneous solution.

Typically the following procedures are used to prepare the samples for analysis:

3.3.2 **For control and 0.01 ppm fortifications**, filter the solution through a syringe filter (a 0.5 micron Fluoropore disc fitted to 1.0 mL disposable plastic syringe). Transfer the sample solution (3.3.1) with a glass disposable pipette to the syringe, discard the initial 100- 200 µL of the filtrate and collect the filtrate (about 1-2 mL) into an injection vial.

3.3.3 **For 0.1 ppm fortifications**, take 1 mL of the sample solution (3.3.2) and dilute to 10 mL with **solvent mixture III**. Sonicate and vortex to ensure a homogeneous solution. Filter the solution into the injection vial using the procedure above.

For 1.0 ppm fortifications, take 1 mL of the sample solution (3.3.1) and dilute to 100 mL with **solvent mixture III**. Sonicate and vortex to ensure a homogeneous solution. Filter the solution into the injection vial using the procedure above.

The sample is ready for injection.

A flow chart of the analytical procedure is presented in Figure 3

3.4 Moisture Determination

An example of a moisture determination procedure is provided in the validation report (page 16 of this report).

3.5. Instrumentation

Suggested LC-MS/MS Operating condition:

Instrument:	PE Sciex API 3000 Biomolecular Mass Analyzer		
Inlet [HPLC System]:	PE Series 200 Micro Pump system with Series 200 Autosampler		
Data System:	MassCrom 1.1		
Column:	Inertsil ODS-3 5µ, 100 X 2.1 mm, [Metachem , Serial No. 9K150217]		
Injection:	Typically 20 µL		
Mobile Phase: [Gradient]	A = water with 4 mM ammonium formate and 0.1% formic acid B = methanol with 4 mM ammonium formate and 0.1% formic acid **Time (min.)** **Composition** 0.0 80% A + 20% B 0.1 10% A + 90% B 0.6 10% A + 90% B 2.6 80% A + 20% B 2.7 80% A + 20% B Run every 5 minutes		
Flow Rate:	400 µL/minute		
	BAS 510 F	**1-(4-Chlorophenyl)-2-aminobenzene**	**2-Chloronicotinic acid**
Expected Retention Times	2:12 minutes		1.47 minutes
Transitions:	343→140		158→122
Ionization Mode:	Positive ion for all analytes; APCI with heated nebulizer (400°C)		

NOTE:

1. The equipment listed was used for method development and validation.Other equivalent hardware may be used. The use of a guard column is optional.

2. The recommended instrument parameters were found to be optimal for the instrument used for the method validation. The exact values used must be optimized for each instrument.

3. The recommended chromatographic systems were found to be optimal for the types of instrument used for the method validation. Different chromatographic systems might be necessary to be developed for different type of instrument.

3.6 Calibration Procedures

Calculation of results is based on peak area measurements using a calibration curve. The standard curve is obtained by direct injection of 20 µL of the mixed BAS 510 F, 2-Chloronicotinic acid, and 1-(4-Chlorophenyl)-2-aminobenzene standards for LC-MS/MS in the range of 0.5 pg/mL to 5.0 pg/mL. In a given injection run, the same volume is used for all samples and standards. Typical standard amounts injected on-column range as follows: 12.5, 25.0, 50.0 and 100 pg.

Prepare calibration curves by plotting the peak area (monitoring transitions m/z 343→140, 158→122, and 204→169 for mixed BAS 510 F, 2-Chloronicotinic acid, and 1-(4-Chlorophenyl)-2-aminobenzene, respectively) versus the weight of mixed BAS 510 F, 2-Chloronicotinic acid, and 1-(4-Chlorophenyl)-2-aminobenzene, respectively using a linear least squares working curve in the form $y = bx + c$.

Establish the stability of the detection response by injecting several concentrations of standards. For analysis, alternate samples and standards. For each injection set, the set should begin and end with standard injections, and each standard level should be injected at least in duplicate.

Note: It is advisable to "stabilize" on column retention time of theanalytes before injecting the first sample of an analytical series.

3.7 Limit of Quantitation and Limit of Detection

The limit of quantitation is defined as the lowest fortification level successfully tested. The limit of quantitation is 0.01 ppm for BAS 510 F and its metabolites, 2-Chloronicotinic acid, and 1-(4-Chlorophenyl)-2-aminobenzene. The limit of detection has not been determined, but the lowest standard for each analyte in calibration curve has good detectability (signal to noise ratio greater than 3:1).

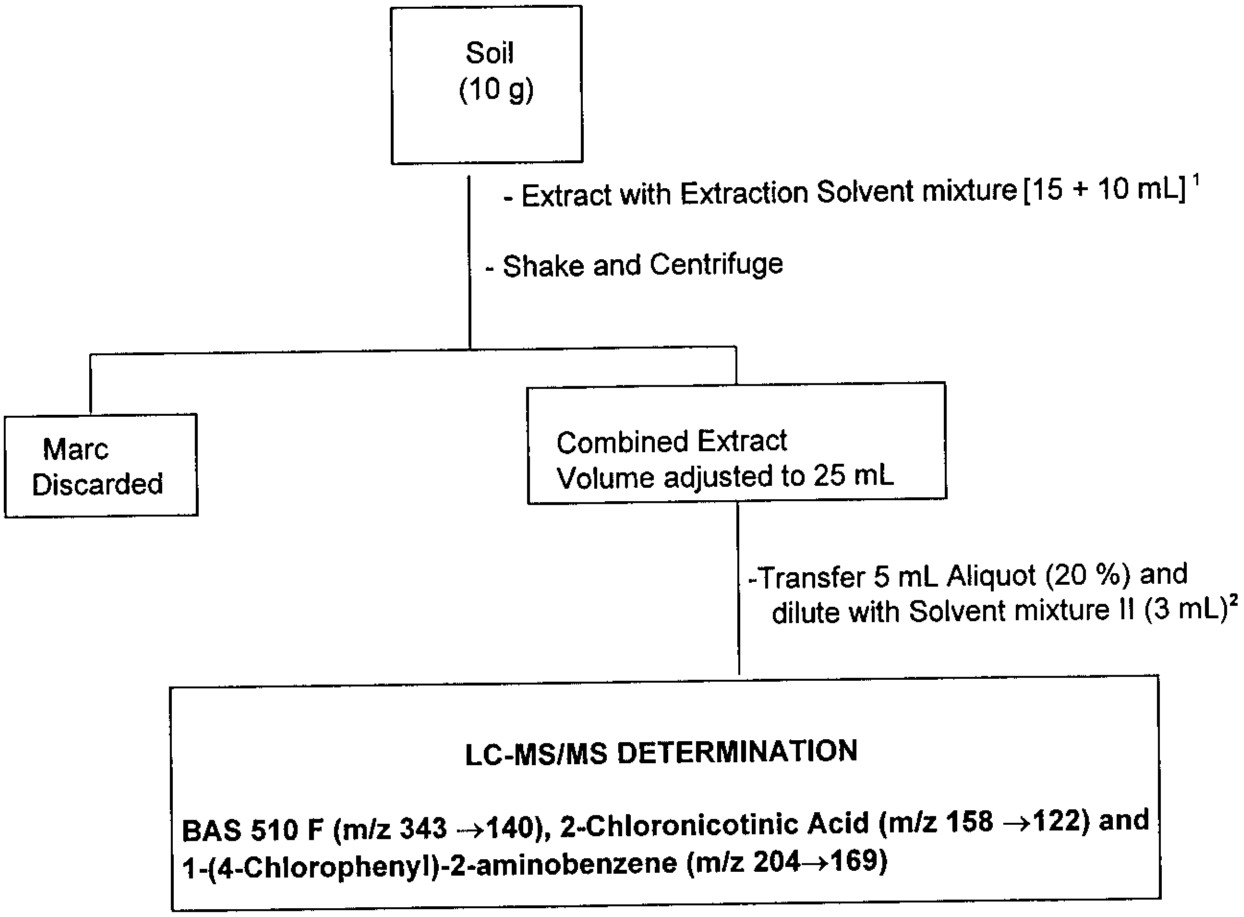
4. Calculation of Results

Calculation of results is based on peak area measurements. A typical calculation to determine the residues of BAS 510 F and its metabolites are shown in page 19 (Figure 2) of this report.

5. Time Requirement for Analysis

The time required for a set of 8 samples (either 6 fortified and 2 controls or 5 treated samples, 2 fortified and 1 control) is approximately 6 person-hours, or 1 calendar day, provided that no special problems arise, such as matrix interference.

Figure 3. Flow Diagram for Analytical Method No. D0004 in Soil (BAS 510 F, 2-Chloronicotinic acid and 1-(4-Chlorophenyl)-2-aminobenzene)



[1] Extraction Solvent Mixture:
First Extraction: Methanol
Second Extraction: Water-Methanol (50:50, and v/v)

[2] Solvent Mixture II: Water with 0.1 % formic acid and 4mM ammonium formate