VALIDATION OF BASF METHOD No. D0903:

I. INTRODUCTION AND SUMMARY

A. PURPOSE OF STUDY

BAS 700 F is a new fungicide that will be used for cereal crops, legumes, soybean, cotton, peanut, pomefruit and root and tuber in the US, Canada, Australlia and Europe. For registration of this fungicide and for establishing the DT50/90 values from field dissipation studies for these use patterns, a residue analytical method with a limit of quantitation of 0.001 mg/kg for the active ingredient and its metabolites in soil was developed. This study was conducted to validate BASF Analytical Method D0903. Recovery ranges and standard deviations were determined from fortified control soil samples. Recoveries of all analytes were determined in two different soil types.

II. MATERIALS/METHODS

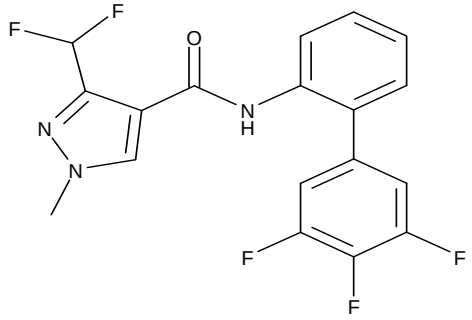
A. TEST AND REFERENCE SUBSTANCES

Fortification Compound

BAS 700 F

Reg-No.: 5094351
Chemical name: 3-(difluoromethyl)-1-methyl-N-(3',4',5'-trifluoro[1,1'-biphenyl]-2-yl)-1H-pyrazole-4-carboxamide

Structural formula:

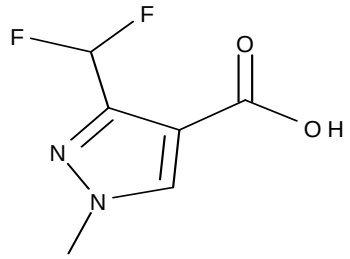


Empirical formula: $C_{18}H_{12}F_5N_3O$
Molecular weight: 381.31 g/mol
Lot no.: L80-28
Purity: 99.7%
Stability: April 01, 2010; stored room temperature or cooler

II. MATERIALS/METHODS (Continued)

M700F001

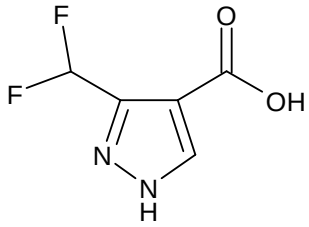
Reg-No.: 5069089
Chemical name: 3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxylic acid
Structural formula:



Empirical formula: $C_6H_6F_2N_2O_2$
Molecular weight: 176.1 g/mol
Lot no.: L80-68
Purity: 99.2%
Stability: August 01, 2010; stored at +4 °C or cooler

M700F002

Reg-No.: 5435595
Chemical name: 3-(difluoromethyl)-1H-pyrazole-4-carboxylic acid
Structural formula:



Empirical formula: $C_5H_4F_2N_2O_2$
Molecular weight: 162.1 g/mol
Lot No.: L80-20
Purity; 99.2%,
Expiration Date: July 01, 2011; stored at + 4°C or cooler

Reference Standard (used for calibration)

Same as fortification compound (Section 2.1.1)

Standard substances are stored in a freezer (<-5^{0}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.

II. MATERIALS/METHODS (Continued)

Standard Solution Stability

During the course of this study, the stability of fortification and LC-MS/MS calibration standard solutions was examined and the stability information is provided in this report. Test and reference substance solutions were stored in a refrigerator at 4°C and 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. The following table shows the stability of the analytes in various solvent system used within the method.

SOLUTION	STABILITY (DAYS)
Stock solutions of BAS 700 F, M700F001 and M700F002in methanol	95
Fortification solutions and LC-MS/MS calibration standards of BAS 700 F, M700F001 and M700F002, in methanol - water with 0.1 % formic acid (50:50, v/v)	34

Soil Extract Stability

During the course of a terrestrial dissipation study (**Reference 1**), the stability of the test and reference substances in the soil extracts was examined and the stability information is provided in this report. To demonstrate the stability of all analytes in soil matrix extracts, two samples from fortified matrices were used. The samples were analyzed at the day of extraction and were stored in a refrigerator. These extracts were re-analyzed using method D0903 after certain days of storage (see table below). The results were compared to the initial analysis (zero day) to establish the stabilities. The following table shows the stability of the analytes in soil extracts in different solutions used within the method.

Extracts	STABILITY (DAYS)[1]
Soil extract containing BAS 700 F, M700F001 and M700F002, in methanol - water (50:50, v/v)	7

II. MATERIALS/METHODS (Continued)

B. TEST SYSTEM

The test systems consisted of untreated soil samples obtained from trial sites of various field dissipation studies conducted in the U.S. These soils were used to validate method D0903. The test systems and their residue control numbers (RCN) and the locations are listed below.

Study No. (Location)	Sample No (original Study)[1]	Sample Description
319651 Manitoba	R0803820006	Clay loam; 24-30" depth
347450 California	R0805320014	Sandy loam; 0-3" depth

Reference 1 and 2

C. SAMPLE STORAGE AND HANDLING

Bulk soil samples received from the field are homogenized with dry ice using a Fitzmill (hammermill) and stored frozen (<-5°C) before analysis.

D. EXPERIMENTAL DESIGN

Control soil samples were fortified by applying standard solutions directly to the soil prior to extraction with a volumetric pipet. The fortified control samples were analyzed to determine the recoveries of each analytes.

A total of two validation sets were conducted with two different types of soil. Each validation set was consisted of a reagent blank, two unfortified soil controls, 5 control soil samples fortified at the LOQ (0.001 ppm) and 5 control soil samples fortified at 10 times LOQ (0.01ppm). A total of 13 samples per soil type were used in the study. All of these sets were subsequently analyzed with the method.

E. METHOD OF ANALYSIS

BASF Analytical Method D0903 was developed to determine the residues of B BAS 700 F and its metabolites M700F001, and M700F002 in soil matrices using LC-MS/MS. The method was designed to determine the residues as individual analyte and will be used for the residue analysis of soil samples collected from soil dissipation studies.

VI. REFERENCES

1: J. Jordan and M. Saha (2009): in progress, TERRESTRIAL FIELD DISSIPATION OF BAS 700 F FOLLOWING APPLICATIONS OF BAS 700 AC F IN ORCHARD AND VINEYARD USE PATTERNS; BASF Corporation, Research Triangle Park, North Carolina; Study 347450 (BASF Registration Document Number 2009/7006033)

2: J. Jordan and M. Saha (2009): in progress, TERRESTRIAL FIELD DISSIPATION OF BAS 700 F FOLLOWING APPLICATIONS OF BAS 700 AC F IN LEGUMES; BASF Corporation, Research Triangle Park, North Carolina; Study 319651 (BASF Registration Document Number 2009/7006030).

3: S. Perez and R. Perez (2009): Independent Laboratory Validation of BASF Analytical Method D0903: "Determination of BAS 700 F (Reg. No. 5094351) and its Metabolites, M700F001 (Reg. No. 5069089) and M700F002 (Reg. No. 5435595) in Soil at LOQ 0.001 mg/kg"; ADPEN Laboratories Inc., Jacksonville, Florida; Study 374091 (BASF Registration Document Number 2009/7003274).

VII. COMMENT FROM INDEPENDENT LABORATORY VALIDATION

In summary, the ILV was completed successfully on the first trial. Therefore, BASF Analytical Method D0903 is suitable for determining residues of BAS 700 F and its two metabolites, M700F001 and M700F002, in soil down to a level of 0.001 mg/kg (ppm). The method is well-written and contains a fair amount of comments to guide the analyst through the procedure for the first time. Recommendations are presented below and also incorporated in the final report in analytical method Section (See Technical Procedure Section 7, potential problem).

Recommendations:

1. It is recommended to establish suitable equilibrium time at the end of the HPLC method to produce consistent analyte retention times. Comparable HPLC systems may vary in the time necessary to equilibrate between sample injections.

2. Keep aware of instrument sensitivity that may lessen due to numerous sample injections. Regular cleaning of the mass spec will help insure enough sensitivity to comply with method specifications for the lowest standard to have a signal to noise ration of 3:1.

Figure 1. Flow Diagram of Analytical Method No. D0903 in Soil

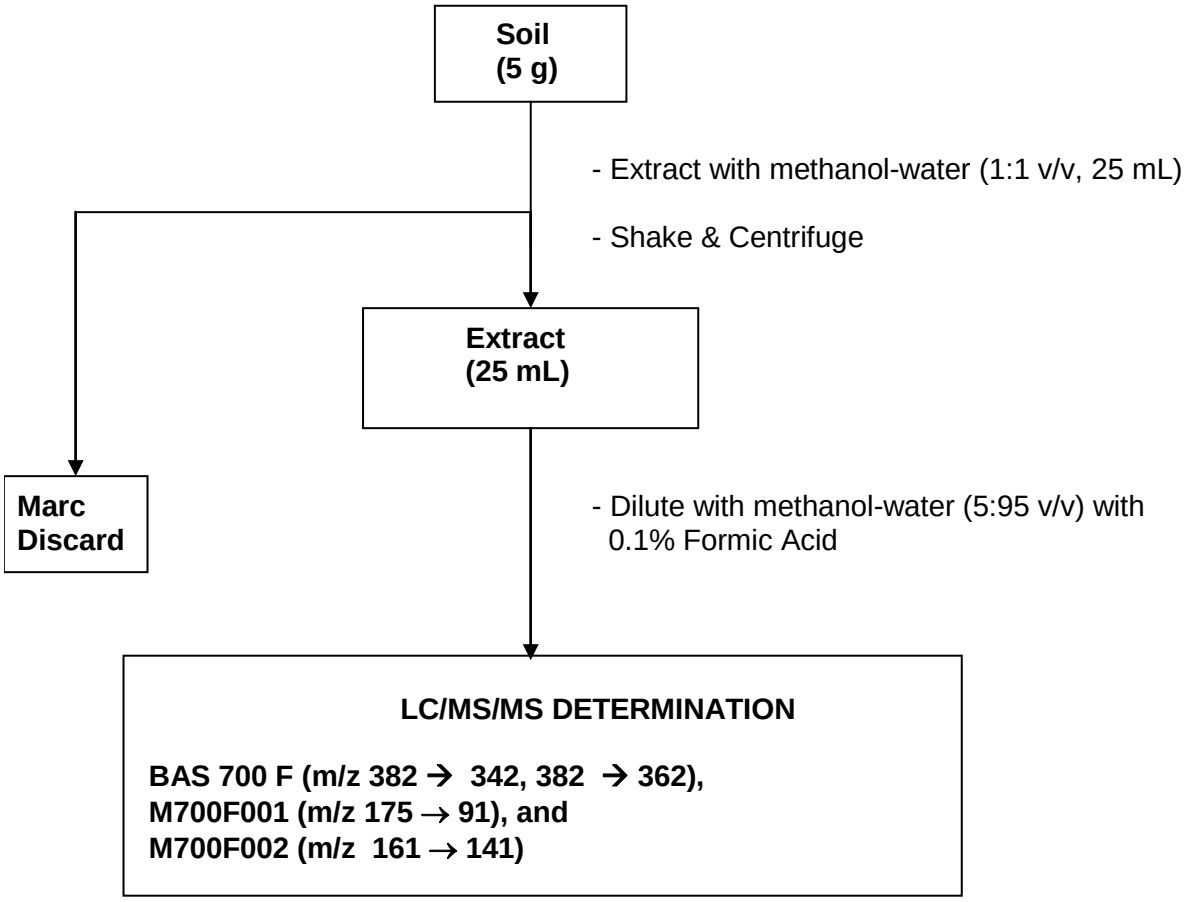


Figure 2. Typical Recovery Calculation (BASF Method D0903)

Sample Number 369563-1F Control soil samples fortified with 0.001 ppm of BAS 700 F, and its metabolites, M700F001, M700F002

Sample Number 369563-1B, C: Controls unfortified, Average (ppm) of two controls were used to subtract the control value for fortifications.

Calculations are shown only for BAS 700 F.

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

Residue calculation for BAS 700 F for the Sample Number 369563-1F**:**
Intercept A = 22.6551
slope B = 85,058.9
correl.coeff. = 0.9998

$$\text{BAS 700 F Found (ng/mL)} = \frac{\text{(peak area – intercept)}}{\text{Slope}}$$

Peak Area = 9002

$$\text{BAS 700 F Found (ng/mL)} = \frac{(9002 - 22.6551)}{85{,}058.9473} = 0.105566$$

Residue (ppm of BAS 700 F) =

$$\frac{\text{BAS 700 F Found (ng/mL) x Final Volume (mL)}}{\text{Sample Weight (g) x } A_F \text{ x 1000 (to convert to ppm)}}$$

Sample Weight = 5 g
Final Volume = 1 mL
Aliquotation Factor (A_F) = 2%

Aliquot Factor = $\frac{\text{Aliquot taken from initial extract (0.5 mL)}}{\text{Total Extraction Volume (25 mL)}} = 0.02$ (2% of original extract)

$$\text{Residue (ppm of BAS 700 F) (wet weight basis)} = \frac{0.105566 \times 1.0 \text{ mL}}{5.0 \text{ g} \times 2\% \times 1000} = 0.0010556 \text{ ppm}$$

Net Residue (ppm of BAS 700 F) = Residue (ppm of BAS 700 F) - Residue in Control (ppm)

$$\text{Recovery of BAS 700 F (\%)} = \frac{\text{Residue (ppm of BAS 700 F) - Residue in Control (ppm)}}{\text{Amount Fortified (ppm)}} \times 100$$

Amount Fortified = 0.001 ppm
Residue in Control = none detected

$$\text{Recovery of BAS 700 F (\%)} = \frac{0.0010556}{0.001} \times 100 = 106\%$$

Full computer/calculator precision in any intermediate calculations is used and the final values are only rounded for reporting purpose. Percent recoveries of all other analytes were calculated in similar fashion.

1. INTRODUCTION

1.1 Scope of the method

BAS 700 F H is a new fungicide that will be used for cereal crops, legumes, soybean, cotton, peanut, pomefruit and root and tuber in the US and Europe. For registration of this herbicide and for establishing the DT50/90 values from field dissipation studies for these use patterns, a residue analytical method with a limit of quantitation of 0.001 mg/kg for the active ingredient and its metabolites in soil was developed.

2. MATERIALS

Standard substances are stored in a freezer 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 Substance

2.1.1 Fortification Compound

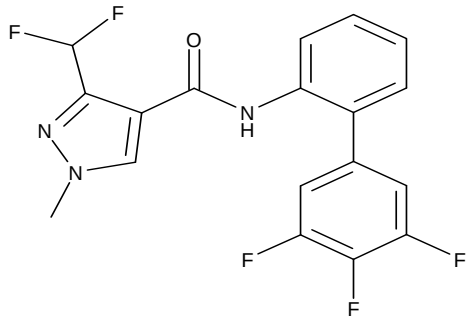
Standard substances are stored in a freezer until use. Information on the characterization of these substances is available from BASF and is located at the Landwirtschaftliche Versuchsstation der BASF, Limburgerhof, Germany.

BAS 700 F

Reg-No.: 5094351

Chemical name: 3-(difluoromethyl)-1-methyl-N-(3',4',5'-trifluoro[1,1'-biphenyl]-2-yl)-1H-pyrazole-4-carboxamide

Structural formula:

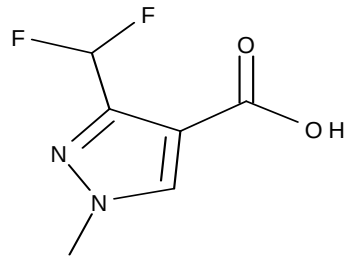


Empirical formula: $C_{18}H_{12}F_5N_3O$
Molecular weight: 381.31 g/mol

2. Materials (Continued)

M700F001 (Reg.no. 5069089)

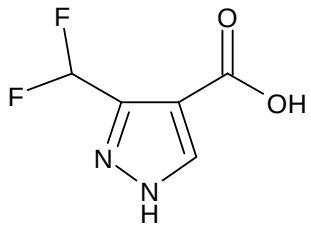
Reg-No.: 5069089
Chemical name: 3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxylic acid
Structural formula:



Empirical formula: $C_6H_6F_2N_2O_2$
Molecular weight: 176.1 g/mol

M700F002 (Reg.no. 5435595):

Reg-No.: 5435595
Chemical name: 3-(difluoromethyl)-1H-pyrazole-4-carboxylic acid
Structural formula:



Empirical formula: $C_5H_4F_2N_2O_2$
Molecular weight: 162.1 g/mol

2.1.2 Reference Standard (used for calibration)

Same as fortification compound (section 2.1.1)

BASF has retained a reserve sample of these chemicals, and has documentation at the BASF Agricultural Products Center, Research Triangle Park, North Carolina.

2. Materials (Continued)

2.2 Equipment -- Suggested Sizes/Suppliers, Manufacturers

Equipment	Size, Description	Manufacturer/ Supplier	Catalog Number
Balance, Analytical	Model AT100	Mettler	
Balance, Top Loading	Model PM 4800	Mettler	
Bottle, Amber glass	Qorpak , 2 oz, 4 oz and 8 oz with Teflon®-lined screw cap	Qorpak	
Centrifuge	Refrigerated Centrifuge Model CS-6KR	Beckmann	
Cylinder, Graduated	Various sizes	Various	
Flask, Erlen Meyer	24/40, 1000 mL	Various	
Flask, Volumetric	100, 50, 25 ,10 and 5 mL	Various	
Lab shaker	EB SM - 30 B	Edmund Bühler (Johanna Otto GmbH) 72379 Hechingen	
MicroMan pipettes	10-1000 µL	Gilson	M-25,M-50,M-250 M-1000
Multitube Vortexer	VX-2500	VWR	58816-116
Pipet, Volumetric	0.5, 1-10, 25 mL	Various	
Pasteur Pipet, disposable	various size	VWR	
Pipet tips	polypropylene	Matrix Inc.	196-205
Syringe Filter, Acrodisc® CR, 0.45 um PTFE membrane	13 mm	Pall Gelman Laboratory	Part No. 4543
PP tubes with srew cap and conical bottom	50 mL	Greiner Bio-one Cellstar® PP-Test tubes	Catalog No. 227 261
Spatula		Various	
Stopper, Teflon®	24/40	Various	
Vials, Amber Borosilicate	8 and 40 mL	VWR	224984 and 15900-018
Votex mixer	Genie 2	Fisher Scientific Co	12-812

2. Materials (Continued)

Equipment	Size, Description	Manufacturer/ Supplier	Catalog Number
LC/MS/MS	4000 Q Trap Mass Analyzer	PE Sciex	
Moisture analyzer	Toledo HR83	Mettler	

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
Methanol	High Purity	B & J	230-4
Water	High Purity	B & J	365-4

2.3.2 Solvent Mixtures and their Preparation

Solvent Mixtures	Method Step
Solution I: Methanol -water, 50:50, v/v Add 500 mL of methanol and 500 mL of water into a 1L Erlenmeyer flask and mix well to ensure complete homogeneous solution.	3.2.3
Solution II: methanol-water with 0.1% formic acid (5:95, v/v) Add 1.0 mL of formic acid (98 %) into a 1L volumetric flask. Add 50 mL of methanol and dilute to the mark with water and mix well to ensure a complete homogeneous solution.	3.5

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

2.4 Standard Solutions and their Storage Stability

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 method validation 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 solution of BAS 700 F, M700F001, and M700F002 (1 mg/mL):

Prepare a 1.0 mg/mL stock solution individually by weighing an appropriate amount of each analyte into a volumetric flask. Dissolve with appropriate solvent as described below and dilute to mark.

For example, to prepare a 10 mL of 1.0 mg/mL stock solution of BAS 700 F in methanol, weigh 10 mg of BAS 700 F into a 10 mL volumetric flask. Dissolve and dilute to mark with methanol. Sonicate and vortex to ensure a complete homogeneous solution. The stock solutions for all other analytes are made in a similar fashion. The solvent used for stock solutions for individual anlaytes methanol.

2.4.2.2 Mix Standards for Fortifications

Prepare mixed standard solution for fortification by combining stock solutions of each analyte (2.4.2.1) in a volumetric flask using the following scheme. Dilute to the mark with appropriate solvents as specified in the table below and vortex to ensure a complete homogeneous solution. Preparation of fortification solutions:

Take stock solution	Volume (mL)	Dilution with Solution I to final volume of [mL]	Concentration mix solution
BAS 700 F, M700F01 and M700F02	1.0 mL each analyte solution	10	100 µg/mL

Take solution of mix standard solution	Volume (mL)	Dilution with Solution I to final volume of [mL]	Concentration
100 µg/mL	1	10	10 µg/mL
10 µg/mL	1	10	1 µg/mL
1 µg/mL	2.5	25	0.1 µg/mL

Solution I: Methanol-water (1:1, v/v)

2. Materials (Continued)

2.4.2.3 Calibration Standard Solutions for LC/MS/MS Analysis

Prepare mixed calibration solution for LC/MS/MS analysis by combining solutions that were prepared in Section 2.4.2.2 in volumetric flasks using the following scheme in the table below. Dilute to the mark with appropriate solvents as specified and vortex to ensure a complete homogeneous solution.

Preparation of calibration standard solutions:

Volume (mL) taken/ Solution used	Dilute to a final volume of (mL) with Solution II	Concentration
2.5 mL of 0.1 μg/mL of mix standard solution in Solution II	50	5 ng/mL
20 mL of 5 ng/mL of of mix standard solution in Solution II	100	1 ng/mL
50 mL of 1.0 ng/mL of mix standard solution in Solution II	100	0.5 ng/mL
50 mL of 0.5 ng/mL of mix standard solution in Solution II	100	0.25 ng/mL
40 mL of 0.25 ng/mL of mix standard solution in Solution II	100	0.1 ng/mL
50 mL of 0.1 ng/mL of mix standard solution in Solution II	100	0.05 ng/mL
20 mL of 0.05 ng/mL of mix standard solution in Solution II	50	0.02 ng/mL

Solution II: methanol-water with 0.1 % Formic acid (5:95, v/v)

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 with dry ice using a Fitzmill (hammermill) and stored frozen (<-5°C) before analysis.

3.2 Weighing and Fortification

Weigh a 5 g or to the nearest tenth of a gram aliquot of the soil sample into a 50 mL - PP tube with screw – cap

For the fortification samples, add volumetrically an appropriate volume of standard solution to the respective control sample by a micro pipet.

Example for preparation of fortified samples (based on 5 g weigh in sample):

Take solution (µg/mL)	Volume spiked of each solution (µL)	Amount spiked (ng)	Spiking Level (mg / kg)
10	50	500	0.1
1	50	50	0.01
0.1	50	5	0.001

3.3 Extraction

3.3.1 Add 25 mL of Solution I (methanol-water, 50:50, v/v) to the poly propylene (PP) tubes containing soil (Section 3.2) using a volumetric pipet. Firmly cap the PP tubes and vortex the samples and shake for 30 minutes on a mechanical shake horizontally at about 225 rpm.

3.3.2 Centrifuge samples at about 3500 rpm for 10 minutes in a centrifuge. Detach the cap. Remove 0.5 mL aliquot of the extract and dilute to 1 mL with Solution II (methanol-water with 0.1% formic acid, 5:95, v/v) and proceed to Section 3.4

NOTE:

- In case of some soil types, it may be necessary to increase the shaking time vortex cycles or to further agitate on a mechanical shaker for 5 minutes. The shaking step, if needed, should be performed in between vortex steps and document in the master sheet.

3.4 Sample Preparation for LC/MS/MS Analysis

3.4.1 For control and 0.001 ppm fortifications samples, were filtered though a syringe filter into a HPLC vial and were directly analyzed.

3.4.3 For higher fortification levels and all residue samples are diluted at the limit of quantitation level (0.001). Any further dilutions are made with Solution II: methanol-water with 0.1 % Formic acid (5:95, v/v) and were filtered though a syringe filter into a HPLC vial prior to LC-MS/MS analysis.

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

3. Analytical Procedures (Continued)

3.5 Moisture Determination

The procedural recoveries will not be corrected for moisture content of the sample .Results of soil analysis are reported on a "dry weight" basis for residue determination. Therefore field treated soil sample weights must be corrected for moisture content by any method the laboratory customarily uses. The moisture determination will be conducted for the treated samples with residue value above LOD. An example of a moisture determination procedure is provided below:

The percent moisture is determined using a automated moisture determination equipment (Mettler Toledo HR83) using the formula below:

$$\text{Percent Moisture} = \frac{\text{Wet Sample Weight (g) - Dry Sample Weight (g)}}{\text{Wet Sample Weight (g)}} \times 100$$

$$\textbf{Residue in ppm (Dry residue)} = \frac{\textbf{Wet Sample Residue (ppm)}}{\textbf{(100 – “Percent Moisture”) / 100}}$$

3. Analytical Procedures (Continued)

3.7. Instrumentation

Suggested LC/MS/MS Operating condition:

Method A:

Instrument:	PE Sciex API 5000 Mass Analyzer				
Inlet [HPLC System]:	Waters Acquity				
Software Version:	Analyst 1.4.2				
Column:	Waters UPLC® HSS T3, 1.8 μ, 2.1 X 100 mm (P/N 186003539)				
Injection:	Typically 10 μL		Column Temperature		50°C
Mobile Phase:	A = Water with 0.1 % formic acid B = Acetonitrile with 0.1 % formic acid				
Gradient	Time (min.)	Flow rate: μL/min.	Gradient Profile	Composition	
				% A	%B
	0.0	600	NA	80	20
	0.8	600	6	50	50
	1.26	600	6	0	100
	1.75	600	6	0	100
	1.76	600	6	80	20
	2.25	600	6	80	20
	BAS 700 F		**M700F001**		**M700F002**
Expected Retention Times	1.68 minutes		0.89 minutes		0.72 minutes
Transitions (m/z):	382 → 362 382 → 314		175 → 91		161 → 141
Ionization Mode:	Turbospray (600°C); Negative for M700F001 & M700F002 Positive for BAS 700 F				

In validation study this method was used only for M700F001 and M700F002 Confirmatory purposes

Method B:

Instrument:	PE Sciex API 4000 Qtrap Mass Analyzer			
Inlet [HPLC System]:	Rheos Ultra 4XTi Pump and CTC Pal Autosampler			
Software Version:	Analyst 1.4.2			
Column:	Waters UPLC® HSS T3, 1.8 μ, 2.1 X 100 mm (P/N 186003539)			
Injection:	Typically 25 μL	Column Temperature: RT (50°C if column heater available)		
Mobile Phase:	A = Water with 0.1 % formic acid B = Acetonitrile with 0.1 % formic acid			
Gradient	Time (min.)	Flow rate: μL/min.	Composition	
			% A	%B
	0.0	500	70	30
	0.1	500	70	30
	0.8	500	50	50
	1.26	500	0	100
	2.95	500	0	100
	3.0	500	70	30
	3.5	500	70	30
	BAS 700 F	**M700F001**	**M700F002**	
Expected Retention Times	~2.3minutes	~1.45 minutes	~1.15 minutes	
Transitions (m/z):	382 → 362 382 → 342	175 → 91	161 → 141	
Ionization Mode:	Turbospray (600°C) Negative for M700F001 & M700F002 Positive for BAS 700 F			

Method C:

Instrument:	PE Sciex API 4000 Qtrap Mass Analyzer			
Inlet [HPLC System]:	Rheos Ultra 4XTi Pump and CTC Pal Autosampler			
Software Version:	Analyst 1.4.2			
Column:	UK-C18 75 X 3.0 mm, 3u			
Injection:	Typically 25 µL	Column Temperature: RT (50°C if column heater available)		
Mobile Phase:	A = Water with 0.1 % formic acid B = Acetonitrile with 0.1 % formic acid			
Gradient	Time (min.)	Flow rate: µL/min.	Composition	
			% A	%B
	0.0	500	70	30
	1.0	500	60	40
	2.0	500	60	40
	3.0	500	30	70
	3.1	500	0	100
	5.9	500	0	100
	6.0	500	70	30
	7.5	500	70	30
	BAS 700 F	**M700F001**	**M700F002**	
Expected Retention Times	~4.1 minutes	~1.3 minutes	~1.1 minutes	
Transitions (m/z):	382 → 362 382 → 342	175 → 91	161 → 141	
Ionization Mode:	Turbospray (600°C) Negative for M700F001 & M700F002 Positive for BAS 700 F			

3.8 Calibration Procedures

Calculation of results is based on peak area measurements using a calibration curve. The calibration curve is obtained by direct injection of BAS 700 F, M700F001, and M700F002 mix standards for LC/MS/MS in the range of 0.02 ng/mL to 0.5 ng/mL. In a given injection run, the same injection volume is used for all samples and standards. Typical standard amounts injected on-column range as follows: 0.5, 1.25, 2.5, 6.25, and 12.5 pg.

Calibration curves are prepared by plotting the peak area versus the weight using a linear least squares working curve in the form of y = bx + c. The transitions monitored are 382 → 362, 382 → 342; 175 → 91; 161 → 141 for analytes BAS 700 F, M700F001, and M700F002 respectively.

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.

3.9 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 0.001 ppm for all analytes. The limit of detection has not been determined, but set at 20 % of the limit of quantitation [e.g. The LOQ is 0.001 ppm and LOD is 0.0002 ppm]. The lowest standard for each analyte in the calibration curve has good detectability (signal to noise ratio greater than 3:1).

4. CALCULATION OF RESULTS

4.1 Principle

Calculation of results is based on peak height or area measurements.

For the procedural recoveries, the sample weight will be considered 5 g in the final calculation of residues [µg/g (ppm)]. 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 recoveries and residues of all analytes in µg/g (ppm) are calculated with the following formulas:

$$\textbf{Residue in ppm} = \frac{\text{Analyte Found (ng/mL) x Final Volume (mL)}}{\text{Sample Weight (g) x } A_F \text{ x 1000 (to convert to ppm)}}$$

$$\text{Analyte Found (ng/mL)} = \frac{\text{(peak area – intercept)}}{\text{Slope}}$$

Sample Weight = 5 g
Final Volume = 1 mL
Aliquotation Factor (A_F) = 2%

$$\textbf{Aliquot Factor} = \frac{\text{Aliquot taken from initial extract (0.5 mL)}}{\text{Total Extraction Volume (25 mL)}} = 0.02 \text{ (2\% of original extract)}$$

$$\textbf{Percent recovery (\%)} = \frac{\text{Residue (µg/g) for [fortified sample - control sample]}}{\text{Amount (µg/g) fortified}} \text{ X } 100$$

5. TIME REQUIREMENT FOR ANALYSIS

The time required for a set of 13 samples (10 fortified, 2 controls and one reagent blank) is approximately 2 person-hours, provided that no special problems arise, such as matrix interference.

6. CONFIRMATORY TECHNIQUES

The method of determination is LC/MS/MS, which is a highly selective and self-confirmatory detection technique. However two ions were monitored during analysis for peak confirmation

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.

The glassware used for the method should be thoroughly rinsed with methanol to prevent contamination.

Peak enhancement could be a potential problem without sufficient sample clean-up. It is highly recommended to perform instrument check routinely during LC/MS/MS analysis for standard peak enhancement or suppression. The instrument check sample is basically prepared by adding known amount of standard to the control matrix at the limit of quantitation (0.001 ppm level). It is recommended to clean the LC/MS thoroughly, if peak enhancement or suppression has been observed. Some of the cleaning procedure included exhaustive cleaning of the hardware, such as skimmer, fused silica for sample introduction, and several gradient systems to wash the column.

It is recommended to establish suitable equilibrium time at the end of the HPLC method to produce consistent analyte retention times. Comparable HPLC systems may vary in the time necessary to equilibrate between sample injections.

Keep aware of instrument sensitivity that may lessen due to numerous sample injections. Regular cleaning of the mass spec will help insure enough sensitivity to comply with method specifications for the lowest standard to have a signal to noise ration of 3:1.

8. SAFETY AND HEALTH CONSIDERATIONS

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