1.0 INTRODUCTION AND SUMMARY

1.1 Scope and Source of the Method

1.1.1 Scope

This method is to be used to determine the residues of BAS 490, BF 490-1 and BF 490-5 in soil. The BAS 490 F residues are first extracted from the soil with methanol; and then the soil is re-extracted with 0.1 N sodium hydroxide to release the acid moieties: BF 490-1 and BF 490-5. The alkaline extract is acidified and partitioned with dichloromethane/ethyl acetate. The partition extract and the methanol extract are concentrated under dry nitrogen separately then combined and further dried to < 1 mL. The residues are analyzed using LC/MS/MS.

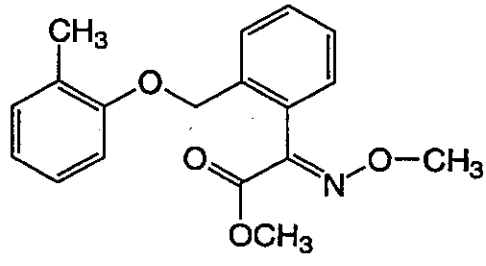
1.1.2 Source

This method was developed at Advanced BioAnalytical Services, 15 Catherwood Road, Ithaca, NY 14850 and BASF Corporation, 26 Davis Drive, Research Triangle Park, North Carolina 27709.

1.2 Test / Reference Substances

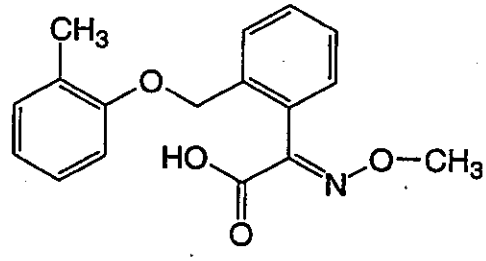
Experimental name:	BAS 490 F	
Chemical name:	methyl-(E)-methoximino[α-(o-tolyloxy)-o-tolyl] acetate	
Empirical formula:	$C_{18}H_{19}NO_4$	
Molecular weight:	313.36	
Melting point:	---	
Appearance:	White crystalline	
Odor:	Mildly aromatic	
Solubility - at 20°C	Water:	2×10^{-4} g/100 g
	Dichloromethane:	93.9 g/100 mL
	methane:	1.49 g/100 mL

Structure:



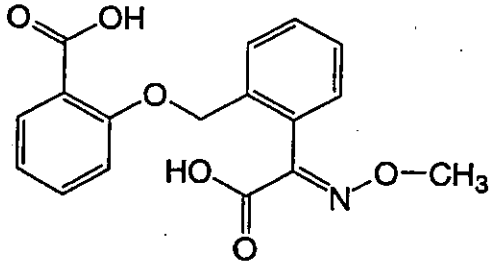
Experimental name: BF 490-1

Chemical name: (E)-methoximino[α-(o-tolyloxy)-o-tolyl] acid
Empirical formula: $C_{17}H_{17}NO_4$
Molecular weight: 299.33
Structure:



Experimental name: BF 490-5

Chemical name: (E)-methoximino[α-(o-tolyloxy)-o-tolyl] diacid
Empirical formula: $C_{17}H_{15}NO_6$
Molecular weight: 329.31
Structure:



1.3 Principle of the Method

Control soil samples were homogenized using a Fitzmill grinder at BASF and were sent to Advanced BioAnalytical Services. Samples were shipped to Advanced BioAnalytical Services from BASF and all samples were received in good condition and frozen on dry ice in insulated boxes. The soil samples were stored frozen at -30 °C. The assay uses 10 g aliquots of soil. ABS sample numbers were assigned in order of receipt by the software package DMLIMS+, Version 4.3.1i (PennComp Inc., Wayne, PA 19087).

BAS 490 F, BF 490-1 and BF 490-5 were extracted from soil using a sequential solvent extraction procedure. Following extraction with 2 x 40 mL of methanol, samples were extracted with 30 mL sodium hydroxide (0.1 N). The alkaline extracts were acidified using 1000 µL of concentrated hydrochloric acid and back-extracted with 3 x 40 mL of 90:10 dichloromethane : ethyl acetate. The methanol extracts and partition extracts are concentrated under dry nitrogen separately to 5 mL and 30 mL, respectively. The extracts are combined and further concentrated to < 1 mL followed by sequential reconstitution in 10 mL of methanol with subsequent addition of 10 mL 8 mM ammonium formate, 0.2% formic acid. The concentrated extracts were then analyzed using ion spray liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) in the selected reaction monitoring (SRM) mode. The desired lower level of quantitation of the analytes in soil was 0.010 ppm.

2.0 MATERIALS AND METHODS

2.1

Equipment	Suggested Sizes/Manufacturer
PE-SCIEX API III^{plus} Triple Quadrupole Mass Spectrometer	PE-SCIEX, Concord, Canada
LC Pump, Shimadzu LC 10-AD	Shimadzu Co., Kyoto, Japan
Autosampler, Waters 717 Plus	Waters, Milford, MA 01757
Alltech On Line Degassing System	Alltech Associates Inc., Deerfield, IL 60015, Cat# 590100
Asahipak ODP LC Column	5 µm particle size, 2 x 100 mm, Keystone Scientific, Bellefonte, PA 16823
Kromasil ODS LC Column	5 µm particle size, 2 x 50 mm Keystone Scientific, Bellefonte PA 16823
Column Heater, Model 7970	Jones Chromatography USA Inc., Lakewood, CO 80228
Harvard Syringe Pump 11	Harvard Apparatus Inc., South Natick, MA 01760
Multi-tube Vortexer	VWR Scientific, West Chester, PA 19380 Cat# 58816-115
Microbalance, Model MT-5	Mettler-Toledo Inc., Hightstown, NJ 08520
Balance, Model FX-300	AND Ltd., Tokyo, Japan
pH Meter, Model 340	Corning Inc., Corning, NY 14830
TurboVap LV Evaporator	Zymark, Hopkinton, MA 01748

	Cat# 43750
NANOpure-UV Water Purification System	Barnstead, Dubuque, IA 52001
Centrifuge:	Dupont RT-6000D Refrigerated or Equivalent
Mechanical shaker:	Eberbach Corp., Ann Arbor, MI 48106 Cat# 6000
Bench Top Shaker	Glas-Col, Terre Haute, IN Cat# 099A S60012
Whatman Autovial Processor, P20	Whatman Inc., Clifton, NJ 07014
Whatman Polypropylene membrane autovials	VWR Scientific, Rochester, NY 14603 Cat# 28297-106
Separatory funnels:	250 mL
Flat bottom flasks:	500 mL
Polypropylene Centrifuge tubes with screw caps	VWR Scientific, West Chester, PA 19380 Cat# 21008-146
Polypropylene Centrifuge tubes with screw caps	VWR Scientific, West Chester, PA 19380 Cat# 21008-725
Volumetric flasks:	10, 25, 50, 100, 250, 500, 1000, 2000 mL
Autosampler vial/caps:	Sun Brokers Inc., Wilmington, NC 28402 Cat# 500 765
Eppendorf Repeater Pipettes	Brinkman Instruments, Westbury, NY 11950, Cat# 22 26 000-6
Eppendorf Repeater Pipette Tips	5 mL, 12.5 mL, 25 mL, 50 mL Brinkman Instruments, Westbury, NY 11950
Pipettes	Cat# M-25, M-50, M-250, P-200, P-1000, P-5000, P-10ML Rainin Instrument Co., Woburn, MA 01888
Pipette Tips	Cat# CP-25, CP-50, CP-250, GPS-96, GPS-1000, C-5000, RC-10ML Rainin Instrument Co., Woburn, MA 01888
Glass Scintillation Vials	20 mL, Wheaton, Millville, NJ 08332

Polypropylene Scintillation Vials	20 mL, Wheaton, Millville, NJ 08332
Repeating Dispenser	Brinkman Dispensette 10-50 mL Brinkman Instruments, Westbury, NY 11950, Cat# 50 10 050-2
Repeating Dispenser	EM Optifix Basic 5, Cat# EM-108033 VWR Scientific, Rochester, NY 14603
Universal REPIPET Dispenser	10 mL, Cat# 53528-168 VWR Scientific, Rochester, NY 14603
Oxford Adjustable Dispenser	3.0 to 10 mL, Cat# 53519-520 VWR Scientific, Rochester, NY 14603
Solvent Filtration Apparatus	Krackeler Scientific, Albany, NY 12201 Cat# 32-00610
Nylon Titan Membrane Filters	0.45 mm, Cat# 74547-NN Scientific Resources Inc., Eatontown, NJ 07724
Volumetric pipettes:	25, 50, 100 mL

Other general laboratory glassware and supplies.

2.2 Reagents and Chemicals — Suggested Source/Preparation

All chemicals may be substituted by that of an equivalent manufacturer and grade of chemical.

Dichloromethane Cat# 300-4	Baxter/Scientific Products, McGaw Park, IL 60085
Methanol, Cat# 230-4	Baxter/Scientific Products, McGaw Park, IL 60085
Water, Cat# 365-4	Baxter/Scientific Products, McGaw Park, IL 60085
Ethyl Acetate, Cat# EX0245-1	EM Science, Gibbstown, NJ 08027
Formic Acid Suprapur Grade, Cat# 11670-1	EM Science, Gibbstown, NJ 08027
Hydrochloric Acid, Cat# 9535-05	J.T. Baker, Danvers, MA 01923
Ammonium Formate, Cat# 09735	Fluka Chemical Corporation, Ronkonkoma, NY 11779
Sodium Hydroxide, Cat# 3722-11	J.T. Baker, Danvers, MA 01923
Acetonitrile, Cat# 015-4	J.T. Baker, Danvers, MA 01923

Ammonium Acetate, Cat# JT0596-11	J.T. Baker, Danvers, MA 01923
Polypropylene Glycol, Average MW=425, Cat# 20230-4	Aldrich Chemical Co., Milwaukee, WI 53233

2.3 Standard Substances and Solutions

2.3.1 Preparation of Solutions

Solutions of 0.1 N sodium hydroxide, 2 mM ammonium acetate, 50:50 methanol : acetonitrile and 8 mM ammonium formate, 0.2% formic acid are assigned a three month expiration date. 90:10 dichloromethane : ethyl acetate was prepared fresh for each experimental run.

1N Sodium Hydroxide: Add 40 g of sodium hydroxide pellets to a 1000 mL volumetric flask and dissolve in HPLC grade water. Allow to cool to room temperature before diluting to the mark with HPLC grade water. Prepare a fresh solution every 12 months.

0.1 N Sodium Hydroxide: Place 200 mL of 1 N sodium hydroxide in a 2000 mL volumetric flask and dilute to the mark with HPLC grade water.

1 M Ammonium Acetate: Add 77 g of ammonium acetate to a 1000 mL volumetric flask and dissolve in HPLC grade water. Dilute to the mark with HPLC grade water. Filter the solution through a 0.45 µM filter with a vacuum flask. Store refrigerated at approximately 4° C and bring to room temperature before use. Prepare a fresh solution every twelve months.

2 mM Ammonium Acetate: Add 1 mL of 1 M ammonium acetate to a 500 mL volumetric flask and dilute to the mark with HPLC grade water and mix thoroughly. Filter the solution through a 0.45 µm filter with a vacuum flask. Store refrigerated at approximately 4° C and bring to room temperature before use.

90:10 Dichloromethane : Ethyl Acetate: Mix 1800 mL of dichloromethane with 200 mL ethyl acetate.

50:50 Methanol : Acetonitrile: Add 500 mL acetonitrile to a 500 mL graduated cylinder. Add 500 mL methanol to a 500 mL graduated cylinder. Combine in a 1000 mL Pyrex bottle and mix thoroughly. Degas by helium sparge.

35:35:30 Methanol : Acetonitrile: 2 mM Ammonium Acetate, Eluent: Add 700 mL of 50:50 methanol : acetonitrile to a 1000 mL graduated cylinder. Add 300 mL of 2 mM ammonium acetate to a 500 mL graduated cylinder. Combine the solutions in a 1000 mL Pyrex bottle and degas by helium sparge.

1 M Ammonium Formate: Add 31.5 g of ammonium formate to a 500 mL volumetric flask and dilute to the mark with HPLC grade water and mix thoroughly. Prepare a fresh solution every twelve months.

8 mM Ammonium Formate, 0.2% Formic Acid: Add 8 mL of 1 M ammonium formate to a 1000 mL volumetric flask and dilute with approximately 800 mL of HPLC grade water. Add 2 mL formic acid to the flask and then dilute to the mark with HPLC grade water and mix thoroughly.

50:50 Methanol : 8 mM Ammonium Formate, 0.2% Formic Acid: Add 100 mL of 8 mM ammonium formate, 0.2% formic acid to a 100 mL graduate cylinder and 100 mL methanol to a separate 100 mL graduated cylinder. Combine solutions and mix thoroughly.

Aqueous Formic Acid (pH 2.5 - 2.75): Place 500 mL of NANOpure water in a 500 mL graduated cylinder. With constant monitoring using a calibrated pH meter add formic acid dropwise until the pH registers a constant pH 2.5 to 2.75. Filter through a 0.45 µm Nylon filter and degas by helium sparge.

30:30:40 Methanol : Acetonitrile: Water: Mix 600 mL of 50:50 methanol : acetonitrile solution with 400 mL NANOpure water. Degas by helium sparge.

31.5:31.5:37 Methanol : Acetonitrile: Aqueous Formic Acid (pH 2.5-2.75): Mix 315 mL of 50:50 methanol : acetonitrile solution with 185 mL Aqueous Formic Acid (pH 2.5-2.75). Degas by helium sparge.

Standards Used:

BASF Code	Lot Number	Purity
BAS 490	39-184-1	99.7%
BAS 490-1	00820-251	100%
BAS-490-5	00436-83	99.6%

Standards supplied by:

BASF Corporation
Agricultural Research Center
P. O. Box 13528
Research Triangle Park, NC 27709

Standards were maintained frozen (<-5 °C) until their use in this study. They were characterized as required by 40 CFR part 160, FIFRA Good Laboratory Practices. Information on the synthesis and subsequent characterization of this substance is available from BASF and is located at BASF Corporation, Agricultural Research Center, Research Triangle Park, North Carolina.

Solutions of standards were refrigerated (approximately +4 °C) during their use in this study. Stock solutions (1 mg/mL) were made fresh every three months. Dilutions of the stock solution were made every three months.

HPLC grade water or equivalent should be used wherever water is called for. Mix

all solutions well. The concentrations of all solutions are based on the free or unionized form of the analytes.

2.3.2 Standard Solutions for Fortifications

BAS 490 F, BF 490-1	1.0 mg/mL, 10.0, 2.0, 0.20 µg/mL in Acetonitrile
BF 490-5	1.0 mg/mL, 10.0, 2.0, 0.20 µg/mL in Methanol

NOTE: Storage containers for 1.0 mg/mL standard solutions and calibration standard solutions are bottles with Teflon-lined screw caps. Storage containers for other stock solutions are Pyrex bottles with polypropylene caps. All storage bottles are foil-wrapped to protect the solutions from light. These concentrations are suggested. Different preparation and concentration schemes may be used and additional standard concentrations may be prepared and used as needed.

Stock Solution Preparation

Separate 1.0 mg/mL solutions of BAS 490 F and BF 490-1 are prepared by weighing 5 mg of the analytical standard solid on a micro balance and transferring to a 5 mL class "A" volumetric flask. Dilution to the mark with acetonitrile yields a 1 mg/mL solution. A 1.0 mg/mL solution of BF 490-5 is prepared by weighing 5 mg of the analytical standard solid on a micro balance and transferring to a 5 mL class "A" volumetric flask. Dilution to the mark with methanol yields a 1 mg/mL solution. The solutions are transferred to scintillation vials, covered with aluminum foil and stored at approximately 4 °C. Fresh solutions are prepared every three months.

2.3.3 Preparation of BAS 490 F, BF 490-1 and BF 490-5 Analytical Standard Working Solutions

A 2 µg/mL Working Solution I of BAS 490 F and BF 490-1 is prepared by transferring appropriate volumes of each of the 1.0 mg/mL stock solutions to a 100 mL volumetric flask. Dilution to the mark with acetonitrile yields a 2 µg/mL solution. A 2 µg/mL Working Solution III of BF 490-5 is prepared by transferring an appropriate volume of the 1.0 mg/mL stock solution to a 100 mL volumetric flask. Dilution to the mark with methanol yields a 2 µg/mL solution. The solutions are transferred to Pyrex bottles, covered with aluminum foil and stored at approximately 4 °C. Fresh solutions are prepared every three months.

A 20 µg/mL Working Solution II of BAS 490 F and BF 490-1 is prepared by transferring appropriate volumes of each of the 1.0 mg/mL stock solutions to a 10 mL volumetric flask. Dilution to the mark with acetonitrile yields a 20 µg/mL solution. A 20 µg/mL Working Solution IV of BF 490-5 is prepared by transferring an appropriate volume of the 1.0 mg/mL stock solution to a 10 mL volumetric flask. Dilution to the mark with methanol to yields a 20 µg/mL solution. The solutions are transferred to Pyrex bottles, covered with aluminum foil and stored at approximately 4 °C. Fresh solutions are prepared every three months.

2.3.4 Standard Solutions for LC/MS/MS Analysis

Calibration curves were prepared from analytical standard solutions (non-extracted)

for each analytical run according to the procedure outlined in Table 1. The concentration of the standards in the calibration curves for the assay were 2.0, 5.0, 10.0, 20.0, 50.0, 100, and 200 ng/mL for BAS 490 F, BF 490-1 and BF 490-5. The standards were prepared by dilution of Working Solutions I to IV with 50:50 methanol : 8 mM ammonium formate, 0.2% formic acid as outlined in Table 1. Analytical standards were placed at the beginning and end of each run and with regular distribution throughout the tray.

Table 1. Analytical Standards Dilution Scheme

Standard	BAS 490F Concentration (ng/mL)	BF 490-1 Concentration (ng/mL)	BF 490-5 Concentration (ng/mL)	Working Solution I (mL)	Working Solution II (mL)	Working Solution III (mL)	Working Solution IV (mL)	Total Volume (mL)*
1	2	2	2	0.025	—	0.025	—	25
2	5	5	5	0.025	—	0.025	—	10
3	10	10	10	0.05	—	0.05	—	10
4	20	20	20	0.1	—	0.1	—	10
5	50	50	50	0.25	—	0.25	—	10
6	100	100	100	—	0.05	—	0.05	10
7	200	200	200	—	0.1	—	0.1	10

* Analytical Standards were diluted to the 10 mL or 25 mL mark with 50:50 methanol : 8 mM ammonium formate, 0.2% formic acid

3.0 ANALYTICAL PROCEDURES

3.1 Preparation of Samples for Extraction

3.1.1 Weigh 10 g of soil sample using a top loading balance into a 50 mL polypropylene centrifuge tube.

3.1.2 As required, fortify control samples with appropriate volumes of fortification solutions.

3.1.3 Fortifications for Procedural Recoveries

Stock solutions containing 1.0 mg/mL BAS 490 F and BF 490-1 in acetonitrile and 1.0 mg/mL BF 490-5 in methanol were prepared separately from those used to prepare the standard curves. These solutions were further diluted and used to prepare the procedural recovery samples.

The recoveries of BAS 490 F, BF 490-1 and BF 490-5 were determined using procedural recovery samples prepared following the procedure outlined below. Acceptable recoveries were defined as not less than 70% and not more than 120% for all samples.

It is recommended that at least two fortifications and one untreated sample (control) are run with each set of samples to monitor method efficiency. Typically, one fortification sample at the limit of quantitation is run along with one fortification sample at a higher level. For each fortified sample, an appropriate volume of standard solution is added to a control soil sample by volumetric pipette. For

example, for a 0.010 ppm fortification, pipette 500 µL of the 0.2 µg/mL standard solution into the control sample. Fortifications are made into the sample before the methanol is added to the sample for the initial extraction.

Soil Procedural Recovery Samples were prepared according to the following procedure:

Procedural Recovery Sample-1 (QC-1), 0.010 ppm: Place 10 g of control soil into a 50 mL polypropylene centrifuge tube. Using an Eppendorf repeater pipette add 500 µL each of 0.2 µg/mL BAS 490 F and BF 490-1 in acetonitrile and 0.2 µg/mL BF 490-5 in methanol. Evaporate the organic solvent under a gentle stream of nitrogen (~2 psi) at 45 °C ± 5 °C for approximately 30 minutes.

Procedural Recovery Sample-2 (QC-2), 0.100 ppm: Place 10 g of control soil into a 50 mL polypropylene centrifuge tube. Using an Eppendorf repeater pipette add 500 µL each of 2 µg/mL BAS 490 F and BF 490-1 in acetonitrile and 2 µg/mL BF 490-5 in methanol. Evaporate the organic solvent under a gentle stream of nitrogen (~2 psi) at 45 °C ± 5 °C for approximately 30 minutes.

Procedural Recovery Sample-3 (QC-3), 0.350 ppm: Place 10 g of control soil into a 50 mL polypropylene centrifuge tube. Using an Eppendorf repeater pipette add 1750 µL each of 2 µg/mL BAS 490 F and BF 490-1 in acetonitrile and 2 µg/mL BF 490-5 in methanol. Evaporate the organic solvent under a gentle stream of nitrogen (~2 psi) at 45 °C ± 5 °C for approximately 30 minutes.

Procedural Recovery Sample-4 (QC-4), 1.00 ppm: Place 10 g of control soil into a 50 mL polypropylene centrifuge tube. Using an Eppendorf repeater pipette add 1000 µL each of 10 µg/mL BAS 490 F and BF 490-1 in acetonitrile and 10 µg/mL BF 490-5 in methanol. Evaporate the organic solvent under a gentle stream of nitrogen (~2 psi) at 45 °C ± 5 °C for approximately 30 minutes.

3.2 Extraction of Residue

3.2.1 Add 40 mL of methanol to the sample and shake on a mechanical shaker for 15 minutes using the mechanical shaker. Shake on the high setting at the maximum speed throughout this procedure. Centrifuge the sample for 5 minutes at 3000 rpm, and decant the supernatant into a centrifuge tube or other collection vessel. Repeat the methanol extraction and decant the supernatant into a centrifuge tube or other collection vessel.

3.2.2 Resuspend the soil in the centrifuge tube with 30 mL of 0.1 N sodium hydroxide and shake for 15 minutes using the mechanical shaker. Centrifuge the extract for 5 minutes at 3000 rpm or until the soil is completely separated from the extract, then decant into a centrifuge tube or other collection vessel.

3.2.3 Resuspend the soil in the centrifuge bottle with 15 mL of NANOpure water and shake for 15 minutes using the mechanical shaker. Centrifuge the extract for 5 minutes at 3000 rpm, or until the soil is completely separated from the extract, then carefully decant into the centrifuge tube or other collection vessel containing the sodium hydroxide extract.

3.2.4 Add 1000 µL of concentrated hydrochloric acid to the centrifuge tube or other collection vessel containing the sodium hydroxide/water extract to lower the pH to ~1. Cap and vortex mix the solution.

3.2.5 Centrifuge the extract for 5 minutes at 3000 rpm to precipitate the humics from the extract.

3.3 Dichloromethane Partition Clean-up

3.3.1 Transfer the acidified extract to a 250 mL separatory funnel (If the supernatant from the centrifugation step 3.2.5 is transferred to a fresh centrifuge tube or other collection vessel, rinse the vessel with NANOpure water and add the aqueous rinse to the separatory funnel).

3.3.2 Extract the aqueous contents of the separatory funnel with 40 mL portions of dichloromethane: ethyl acetate (90:10). Shake the funnel on a mechanical shaker for 2 minutes, and allow the layers to separate. Remove the organic layer into a new centrifuge tube or other collection vessel. Repeat for a total of 3 partitions.

NOTE: The centrifuge step 3.2.5 should have removed all particulates that will cause emulsions. During shaking of the separatory funnels, soils with a high organic content may form emulsions which will not disperse on standing. When this occurs, remove the organic layer and the emulsified layer into a centrifuge tube and centrifuge at 3000 rpm for 3 minutes. Following centrifugation, transfer the aqueous layer back into the separatory funnel.

3.3.3 Concentrate the combined dichloromethane fraction to approximately 30 mL using a TurboVap or rotary evaporator with water bath at 45° C ± 5 °C.

3.3.4 Concentrate the methanol fraction to 20 mL using a TurboVap or rotary evaporator with water bath at 45° C ± 5 °C. Combine the fractions in one centrifuge tube. Concentrate the fraction to < 5 mL but avoid concentrating to dryness.

3.3.5 Combine the methanol and dichloromethane fractions and concentrate to < 1 mL but avoid going to dryness, using a TurboVap or rotary evaporator with water bath at 45° C ± 5 °C. Add sufficient methanol to the 10 mL mark on the centrifuge tubes.

3.4 LC/MS/MS Analysis sample preparation

3.4.1 Add 10 mL 8 mM ammonium formate, 0.2% formic acid, vortex mix and filter the suspensions using polypropylene membrane autovials.

NOTE: The reconstitution process for procedural recovery and study samples is a two-step process. The samples are initially diluted to 10 mL with methanol and then a further 10 mL of 8 mM ammonium formate, 0.2% formic acid is added before filtration. Addition of 10 mL of 8 mM ammonium formate, 0.2% formic acid to methanol will yield a final volume of >19.2 mL. All results from procedural recovery and study samples were multiplied by 0.96 to compensate for this volume compression.

3.4.2 Transfer 100 µL of each sample to a labeled WISP autosampler vial for LC/MS/MS analysis. For the 1.00 ppm Procedural Recovery samples, pipette 225 µL of the reconstitution solution into a WISP vial and add 75 µL of the 1.00 ppm Procedural Recovery solution and vortex mix.

3.4.3 Prepare a 10 ng/mL post-extract spike solution by adding 25 µL of the 2 µg/mL Spiking solution of BAS 490 F and BF 490-1 and 25 µL of the 2 µg/mL Spiking solution of BF 490-5 to a 5 mL volumetric flask and diluting to the mark with control sample extract. Vortex mix and transfer 100 µL to a labeled WISP autosampler vial for LC/MS/MS analysis.

3.5 Instrumentation Common to LC/MS/MS Methods 1 and 2

Instrument:	PE SCIEX API III^{plus} Triple Quadrupole Mass Spectrometer
Degasser:	Alltech On-Line Degassing System
HPLC pump:	Shimadzu LC-10AD
Autosampler:	Waters WISP 717^{Plus}
Column Heater:	Jones Chromatography, Model 7970
Data System:	Macintosh Quadra 900 Data System

Other equivalent hardware may be used.

3.6 Typical Operating Conditions for Lower Organic Content Soils (Method 1)

LC Column	2 mm x 100 mm Asahipak ODP, 5 µm particle size, 200 Å pore size
Mobile Phase	35:35:30 Methanol : Acetonitrile : 2 mM Ammonium Acetate (Helium sparged)
Column Temperature	45 °C
Flow Rate	200 µL/min.
Initial Pressure	60 bar
Injection Volume	3 µL
Autosampler Wash	Acetonitrile (Helium sparged)
Run Time	6.30 minutes
MS Acquisition Time	5 minutes

The time difference between run time and MS acquisition time is explained in Section 3.10.1

MS Mode	Negative Ion for 2.8 minutes then Positive Ion for 2.2 minutes.
Ion Spray Voltage	-3500 V for 2.8 minutes then +4600 V for 2.2 minutes.
Declustering Potential	-15 V for 2.8 minutes then 21 V for 2.2 minutes
Curtain Gas	U.H.P. Nitrogen @ 1.2 Liters/minute
Nebulizer Gas	Nitrogen @ 70 psi
Turbo Ion Spray Temperature	300°C
Turbo Ion Spray Auxiliary Gas	8 Liters/minute

Dwell Time	100 ms for 2.8 minutes then 350 ms for 2.2 minutes.
Collision Gas	Argon at 1.9×10^{15} atom/cm^2
Collision Energy	-4 eV for 2.8 minutes then 11 eV for 2.2 minutes.

The infusion full-scan single MS positive ion spray mass spectrum for BAS 490 F demonstrated abundant ions for the protonated molecules ($[M+H]^+$) at m/z = 314.1 (Figure 1). The full-scan single MS negative ion spray mass spectra for BF 490-1 and BF 490-5 demonstrated abundant ions for the deprotonated molecules ($[M-H]^-$) at m/z = 298.2 and 328.2, respectively (Figure 1). The full-scan MS/MS mass spectrum of BAS 490 F showed product ions at m/z = 116, 131, 132, 162, 206, 222, 223, 235, 238, 267 and 282 with a relative ion abundance >10% from the $[M+H]^+$ precursor ion at m/z = 314.1 (Figure 2). The full-scan MS/MS mass spectrum of BF 490-1 showed product ions at m/z = 102 and 222 with a relative ion abundance >10% from the $[M-H]^-$ precursor ion at m/z = 298.2 (Figure 2). The full-scan MS/MS mass spectrum of BF 490-5 showed product ions at m/z = 102 and 252 with a relative ion abundance >8% from the $[M-H]^-$ precursor ion of m/z = 328.1 (Figure 2). Plausible MS/MS bond cleavage pathways for each compound are shown in Figure 3. The following selected reaction monitoring (SRM) transitions of the deprotonated molecules BF 490-1 and BF 490-5 and the protonated molecule of BAS 490 F were used to quantify the analytes in soil (Figures 1 to 3):

Analyte	Transition monitored	Retention Time
BF 490-5	m/z = 328.2 → m/z = 252.0	1.75 min.
BF 490-1	m/z = 298.2 → m/z = 222.0	1.83 min.
BAS 490 F	m/z = 314.2 → m/z = 116.0	3.22 min.

To achieve the desired lower level of quantitation the use of Turbo Ion Spray was determined to be vital. In contrast to many other test compounds, it was found that standard operation at 500 °C would initially increase the response observed for BAS 490 F, BF 490-1 and BF 490-5, but then the response rapidly decreased with time. Optimization of the Turbo Ion Spray temperature showed that the response for BF 490-1 and BF 490-5 optimized at 350 °C and then dropped off rapidly and BAS 490 F optimized around 400 °C. To ensure sensitivity without subsequent reduction in signal for BF 490-1 and BF 490-5 from overheating, the Turbo Ion spray temperature was maintained at 300 °C for all experiments.

During method development, the presence of formic acid in the reconstitution solvent was observed to be vital for maintenance of the retention and peak shape of BF 490-1 and BF 490-5. The separation of the three analytes was achieved within four minutes.

It was observed that the LC/MS/MS experiments required some system equilibration time because the response for BF 490-5 did not immediately optimize and the baseline in the chromatogram for BAS 490 F started relatively high. Therefore, it is recommended that several test injections of high standards be injected to determine that the observed peak heights are constant. The low analytical standard should also be injected to verify that it is readily seen above the baseline.

3.7 Typical Operating Conditions for Higher Organic Content Soils (Method 2)

A second LC/MS/MS approach, using an acidified mobile phase, where the fragmentation of the protonated molecules was used to provide the basis for the selected reaction monitoring transitions was developed for the determination of BAS 490 F, BF 490-1 and BF 490-5 residues for higher organic content soils.

LC Column	2 mm x 50 mm Kromasil ODS, 5 µm particle size
Mobile Phase	31.5 : 31.5 : 37 Acetonitrile : Methanol : Aqueous Formic Acid (pH 2.5-2.75) (Helium sparged)
Column Temperature	45 °C
Flow Rate	180 µL/min.
Initial Pressure	35 bar
Injection Volume	15 µL
Autosampler Wash	Acetonitrile : Methanol : Water (30:30:40) (Helium sparged)
MS Mode	Positive ion
Ion Spray Voltage	+4000 V
Declustering Potential	+14.3 V
Curtain Gas	U.H.P. Nitrogen @ 2.2 Liters/minute
Nebulizer Gas	Nitrogen @ 60 psi
Turbo Ion Spray Temperature	400°C
Turbo Ion Spray Auxiliary Gas	>8 Liters/minute
Dwell Time	200 ms
Collision Gas	Argon at 2.4 x 10^{15} atom/cm^2
Collision Energy	15.7 eV

The full-scan single MS positive ion spray mass spectra for BF 490-1 and BF 490-5 demonstrated weak ions for the protonated molecules ($[M+H]^+$) of m/z = 300.2 and 330.2, respectively, with ions of much higher abundance corresponding to the ammoniated molecules, sodiated molecules and weaker potassiated molecules (Figure 4). The full-scan MS/MS mass spectrum of the protonated molecule of BF 490-1 showed product ions at m/z = 116, 209, 222 and 235 with a relative ion abundance >10% from the $[M+H]^+$ precursor ion at m/z = 300.2 (Figure 5). The full-scan MS/MS mass spectrum of the protonated molecule of BF 490-5 showed product ions at m/z = 116, 168, 192 and 236 with a relative ion abundance >5% from the $[M+H]^+$ precursor ion at m/z = 330.2 (Figure 5). A plausible MS/MS fragmentation pathway for the most abundant product ion at m/z =116 seen for BF 490-5 and BF 490-1 is shown in Figure 6. The following selected reaction monitoring (SRM) transitions of the protonated molecules of BAS 490 F, BF 490-1 and BF 490-5 were used to quantify the analytes in soil (Figures 4 to 6):

Analyte	Transition monitored	Retention time
BF 490-5	m/z = 330.2 → m/z = 116.0	1.63 min.
BF 490-1	m/z = 300.2 → m/z = 116.0	3.60 min.
BAS 490 F	m/z = 314.2 → m/z = 116.0	6.00 min.

A dummy transition from m/z = 600 to m/z =250 is inserted between BF 490-5 and BF 490-1 to minimize crosstalk in the Q_2 collision cell.

Rigid control of the LC system and experimental conditions is essential. All three

compounds readily form adduct ions, and in the presence of ammonium salts, the base peak in Q1 scans for each compound will be the ammonium adduct. During method development we attempted to use $(M+NH_4)^+$ -> product ion transitions, but encountered severe problems where the peak area increased throughout the analytical run. This directed us to focus on formation of the $(M+H)^+$ ions. Conversion of the LC system and autosampler from running with ammonium formate to running with formic acid takes about three days. Additionally, removal of all ammonium salts from the system leads to extended run times. The presence of ammonium formate in the reconstitution solvent introduces sufficient ammonium salt into the system to maintain reasonable run times without compromising sensitivity. Once the LC/MS/MS system is equilibrated, system sensitivity is verified by injection of a 2 ng/mL standard solution.

LC/MS/MS Method 2 was originally developed using the m/z = 314.2 to m/z =222 transition of BAS 490 F to minimize the potential for cross talk in the Q_2 collision cell. This was the transition monitored during the cross-validation to Oregon Soil 94016 and New York Soil 94015. When the method was applied to the analysis of samples an interfering peak was observed for BAS 490 F. A subsequent tray was acquired where both the m/z = 314.2 to m/z = 222 and the m/z = 314.2 to m/z = 116 transitions were monitored. The m/z = 314.2 to m/z = 116 transition resulted in increased sensitivity together with minimal interference. This transition was used in all subsequent analyses using LC/MS/MS Method 2.

The preceding specifications are suggested and may be altered as needed. Actual use conditions and any changes must be documented.

3.8 Calibration Procedures

Calibrate the mass axis of the instrument by infusion of a 10^{-4} M solution of PPG 425 in 50:50 methanol : water containing 0.1% formic acid, 0.1% acetonitrile and 2 mM ammonium acetate, at a flow rate of 20 µL/min. Mass calibrate at m/z = 94, 251, 326, 384, 442, 558 and 674. For LC/MS/MS Method 1 tune and optimize the instrument by infusion of a 1 mg/mL solution of BF 490-5 at 20 µL/min into 180 µL/min mobile phase via a zero dead volume T-junction by monitoring the change in relative ion abundance of the m/z = 252 product ion. For LC/MS/MS Method 2 tune and optimize the instrument by infusion of a 1 mg/mL solution of BF 490-5 at 20 µL/min into 180 µL/min mobile phase via a zero dead volume T-junction by monitoring the change in relative ion abundance of the m/z = 116 product ion. When the mobile phase flow rate is initiated the sensitivity of the m/z = 116 product ion will optimize as the system temperature equilibrates (approximately 5 minutes). Be aware that tuning with the ester for either method will mean that a high background will occur. This will require a few hours to diminish to an acceptable level before sample analysis.

Peak widths should be approximately 0.6 amu at half-height in both single MS and MS/MS mode.

On each day of analysis, analytical standard solutions were employed as described. In each tray (run) one set of analytical standards was inserted at the beginning and one set was inserted at the end of the tray. Additionally, analytical standards were distributed every one to three samples so that at least three replicates of each calibration standard level are injected per analytical run. Quantitation of all samples was achieved using calibration curves generated from injection of the analytical standard solutions.

Standard curve correlation coefficients were >0.98.

3.9 Sample Analysis

Inject an appropriate amount of sample into the LC/MS/MS system depending on the LC/MS/MS method used.

Directly compare the peak area of unknown samples injected with the standard curve to obtain ng/mL values for BAS 490 F, BF 490-1 and BF 490-5 concentrations in the sample extract.

Bracket every 1-3 samples with standards to check for shifts in sensitivity. If the peak area of the unknown is larger than the highest standard, dilute the unknown appropriately and reinject. Reconstitution solvent blanks are injected at the beginning and end of each analytical tray to test for sample carryover. Standard solutions (10 ng/mL) prepared in control blank soil extract are injected to test for analyte signal suppression.

3.10 Interferences

3.10.1 Sample Matrices

LC/MS/MS Method 1 was suitable for the determination of BAS 490 F, BF 490-1 and BF 490-5 in soils with a low amount of endogenous organic material. However, further application of the method revealed limitations when it was employed for the analysis of extracts from soils with a high organic acid content. The presence of co-extractants led to suppression of BF 490-1 and BAS 490 F which displayed low apparent recoveries. Additionally sample carryover was observed for BF 490-1 and BF 490-5.

Full-scan LC/MS acquisition using LC/MS/MS Method 1 revealed that endogenous soil components co-eluted with BAS 490 F, BF 490-1 and BF 490-5. However, using liquid chromatography in conjunction with tandem mass spectrometry, no co-eluting interferences were observed in the selected reaction monitoring chromatograms. When soil extracts were analyzed in the full-scan acquisition mode some late eluting soil components were observed. When the LC run time matched the MS acquisition time (four minutes) these components co-eluted with BF 490-1 and BF 490-5 from a subsequent injection causing a signal enhancement for those two analytes. A longer LC run time (six minutes) meant that the late eluting components had eluted from the column before injection of the next sample.

A second LC/MS/MS method was developed using an acidic mobile phase where all of the analytes were subsequently monitored as their protonated molecule ions. BF 490-1 was retained relative to co-extractants so suppression was minimized. In comparison to Method 1, the sensitivity for BF 490-1 and BF 490-5 was reduced but was sufficient for the analysis of samples. Sample carryover did not occur.

During the experimental procedure the amount of concentrated hydrochloric acid added was observed to be critical, particularly for soils from the 12-18" depth. Method D9503 stipulated that the post-addition pH of the solution should be less than 2. For California Soil 94017 the addition of 300 µL of concentrated hydrochloric acid lowered the pH to ~1.6. However, for lower depth samples, the apparent recoveries of BAS 490 F were around 60% because of interfering components. Raising the volume of

hydrochloric acid to 500 µL (pH ~ 1.4) solved this and no further difficulties were observed for California Soil 94017 or Oregon Soil 94016. For New York soil 94015 the problem was observed for BF 490-5 where the lower depth recoveries were around 60%. For New York soil procedural recovery samples from 6-12" and 12-18" depths varying amounts of hydrochloric acid were added. It was observed that a greater precipitate of humic acids was observed for additions of 300 µL (pH ~2.6) and 500 µL (pH ~ 1.6) hydrochloric acid but recoveries of BF 490-5 were <70%. Addition of 800 µL (pH ~ 1.3) or 1000 µL (pH ~ 1.1) of hydrochloric acid gave reduced precipitates but experimental recoveries of ~90%.

3.10.2 Other Sources

Other Pesticides:

None known to date

Solvents:

None known to date

3.11 Confirmatory Techniques

No problems with questionable peak identity have been encountered to date. Interference from centrifuge tubes from Lot Numbers ≥ 6000 yielded a peak of high abundance which eluted from the column before BF 490-5. The peak for BF 490-5 was observed as a shoulder on the tail of this much larger peak making quantitation difficult. Re-extraction of these samples for BF 490-5 only, using centrifuge tubes from appropriate Lot Numbers showed peaks for BF 490-5 which were free of any interfering peaks.

3.12 Time Required for Analysis

Using the Method as supplied by BASF, a run of 20 to 50 samples with additional duplicate blanks and eight procedural recovery samples would take four working days from soil weighing to LC/MS/MS analysis. Further investigation of the length and number of steps required for analysis revealed that reduction of shake times to 15 minutes, centrifuge times to 5 minutes and elimination of the fourth set of separatory funnels did not affect procedural recoveries. This meant that an equivalent number of samples could be processed in about two working days.

3.13 Potential Problems

Use of inappropriate centrifuge tubes can lead to interference of BF 490-5.

3.14 Moisture Content

The procedure for the moisture determination utilized oven drying of the soil samples to constant weight. The soils were weighed as 5 g aliquots into glass scintillation vials in batches of 40. The batches of weighed soils were dried at 110 °C for 24 hours in a temperature-controlled oven. Some batches were re-weighed after a second 24-hour drying period to verify that they were dried to constant weight. Results indicated that 24 hours was a sufficient time period at this temperature to dry the soils. Percentage

water was calculated on a dry-mass basis. Details of the procedure are described in Appendix I.

4.0 METHODS OF CALCULATION

4.1 Calibration

All calculations of BAS 490 F, BF 490-1 and BF 490-5 are based on SRM LC/MS peak areas. For LC/MS/MS Method 1, concentrations (ng/mL) of BAS 490 F, BF 490-1 and BF 490-5 in the procedural recovery samples are determined using the slope and intercept values from a weighted quadratic regression (1/y) of the peak area versus concentration data from the calibration standards. For LC/MS/MS Method 2, concentrations (ng/mL) of BAS 490 F, BF 490-1 and BF 490-5 in the procedural recovery samples are determined using the slope and intercept values from a weighted linear regression (1/y) of the peak area versus concentration data from the calibration standards.

Results are reported as ng/mL of BAS 490 F, BF 490-1 and BF 490-5 which are extrapolated to provide % recovery for the procedural recovery samples following correction for change in volume with the two-step reconstitution. Sample residues are reported as ppm of analyte following correction for moisture content and reconstitution volume.

4.2 Analyte in Sample

Convert concentration (ng/mL) interpolated from standard curve to total ng in sample i.e. For 20 mL reconstitution volume (ng/mL x 20)

Convert ng to µg (Divide by 1000)

This is µg obtained from ~10 g of soil:- Divide by measured weight of the soil sample to obtain ppm (µg/g) value for each analyte.

Multiply x 0.96 to compensate for the change in volume during reconstitution (See Section 3.4.1).

To correct for moisture;

Moisture corrected concentration = ppm x [(%Water/100) + 1]

4.3 Calculation of Procedural Recoveries

$$\% \text{ Recovery} = \frac{\text{ppm concentration interpolated from standard curve x 0.96 x 100}}{\text{ppm BAS 490 F, BF 490-1 and BF 490-5 added}}$$

Where 0.96 is the factor included to compensate for the change in volume during reconstitution.

Do not correct treated sample results for either control residues or procedural recoveries.