ANALYTICAL PROCEDURES: The application verification pads were analyzed by method RM-30V. These samples were received in glass jars, and were extracted by adding 100 mL acetone and shaking for approximately 5 minutes. The extract was diluted (1.0 mL into 25 mL) and analyzed using a gas chromatograph with a nitrogen-phosphorous detector (GC/NPD). A copy of this method is included in Appendix II.

The analytical methods used to determine residues in soil were RM-30S-1 (flumioxazin, THPA, HPA, and SAT-482-HA) and RM-30S-2-1 (APF and DAPF). Method RM-30S-1 was modified for flumioxazin, THPA, and HPA analysis only – and this modification (RM-30S-1-1) included weighted linear calibration (non-zero intercept) and analysis of flumioxazin by GC/MS or by GC/NPD (with on-column injection). All of the samples were analyzed by method RM-30S-1, except for the last storage stability set that was only analyzed for flumioxazin. Copies of RM-30S-1 and RM-30S-2-1 (and pages from RM-30S-1-1 describing GC/NPD conditions and weighted linear response calculations) are included in Appendix II.

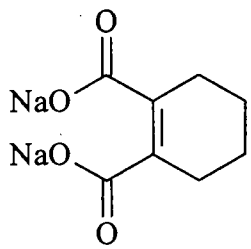
For RM-30S-1, the soil samples are extracted with acetone/1% HCl (9:1, v/v) and with 80 mL acetonitrile/1% HCl (8:2, v/v). The combined extracts are split - with half of the extract partitioned to allow for flumioxazin analysis by GC/MS, and a fourth of the extract cleaned up on a C_{18} cartridge for THPA, HPA, and SAT-482-HA analyses by LC/MS-MS.

The flumioxazin residues are isolated by rotary evaporating the organic solvent and then partitioning the residues from the aqueous solution into hexane. The hexane is removed by rotary evaporation, the residues are dissolved in toluene, and the concentrated extract is analyzed by GC/MS.

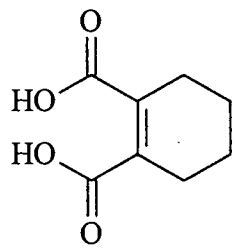
The THPA, HPA, and SAT-482-HA residues are isolated by rotary evaporating the organic solvent, centrifuging the sample to separate the dissolved residues (THPA and HPA), and loading the residues in the supernatant liquid onto a C_{18} cartridge. The cartridge is rinsed with water to remove acid, the THPA and HPA residues are eluted with methanol/water (3:7, v/v; 0.005 M NH_4OOCH), and eluant is analyzed by LC/MS-MS for THPA and HPA. The solids obtained from centrifuging are extracted with methanol/water (3:7, v/v), the mixture is centrifuged, and the supernatant liquid is transferred onto the C_{18} cartridge. The extraction of the solids from centrifuging is repeated with methanol/water (1:1, v/v), the mixture is centrifuged, and the

supernatant liquid is transferred to the cartridge as before. The SAT-482-HA is then eluted from the column with methanol/water (1:1, v/v; 0.01 M Formate Buffer), with analysis by LC/MS-MS. The method Limit of Quantitation (LOQ) was 0.02 ppm and the Limit of Detection (LOD) 0.01 ppm for each analyte. Note that the LOD for THPA is presented as 0.008 ppm in this report.

The THPA standards prepared were actually prepared with the disodium salt of the dicarboxylic acid (THPA·2Na). The molecular weight of THPA·2Na is 214.12 g/mole. The molecular weight of THPA, in the acid form, is 170.16 g/mole. Fortifications and calibration mixtures were all prepared from the disodium salt *without correction to give equivalents of THPA*. The results presented in Appendix VII (Residue Data) are results as THPA·2Na. The concentrations of THPA in both the method validation and in this study were actually 20% less (due to the ratio of molecular weights, 170.16 g/214.12 g) than those presented in the analyses. The results for the residues in the field soil samples have been corrected in the tables to reflect THPA concentrations.



THPA·2Na



THPA

Method RM-30S-2-1 was used for determination of APF and DAPF [dihydro-APF] in soil. In this method, each soil sample is extracted twice with a mix of 40 mL methanol and 10 mL of 0.2 M phosphate buffer (pH 6.5). The combined extracts are transferred to a round-bottom flask, and the mixture is rotary evaporated to remove organic solvent. The residues are partitioned from the resulting aqueous solution into dichloromethane, and the dichloromethane extract is filtered through paper filter. The dichloromethane is removed by rotary evaporation (with octanol added to prevent loss of APF and DAPF), and the residues are then dissolved in toluene and analyzed by GC/MS. Calibration of the GC/MS required using a weighted linear fit for the responses from the linearity standards. [This calibration was also routinely used for calibration of the LC/MS-MS for THPA, HPA, and SAT-482-HA.] For both APF and DAPF, the limit of detection (LOD) was 0.01 ppm and the limit of quantitation (LOQ) was 0.02 ppm.

QUALITY ASSURANCE: Quality assurance measures taken during the analytical portion of this study included, but were not limited to the following:

All analytical standards used in this study were prepared and stored in accordance with Valent SOP's. This requires that standard solutions be kept refrigerated at all times when not in use. It also requires that "working solutions", which are diluted periodically from a stock solution, be calibrated against a "monitoring stock solution".

The following table lists the certification data for the standards used in this study. Certificates of quality are included in Appendix III.

Analytical Standards

Chemical	Standard ID	Certification Date	Percent Purity	Certification
Flumioxazin	AS#1663i	5/2/02	99.2%	Valent USA Corporation
APF	AS#1981b	6/10/02	99.7%	Valent USA Corporation
DAPF	AS#2007a	6/17/02	99.5%	Valent USA Corporation
THPA·2Na*	AS#1987b	6/12/02	99.4%	Valent USA Corporation
HPA	AS#2001a	1/14/02	95.4%	Valent USA Corporation
SAT-482-HA	AS#1994b	7/24/02	99.7%	Valent USA Corporation

* As the disodium salt.

Appendix II

Analytical Methods RM-30V-1, RM-30S-1,
and RM-30S-2-1

VALENT U.S.A. CORPORATION
VALENT TECHNICAL CENTER
DUBLIN, CALIFORNIA

DETERMINATION OF FLUMIOXAZIN IN/ON APPLICATION VERIFICATION PADS
METHOD RM-30V **DATE: May 29, 1999**

INTRODUCTION

This method determines residues of flumioxazin, 2-[7-fluoro-3,4-dihydro-3-oxo-4-(2-propynyl)-2*H*-1,4-benzoxazin-6-yl]-4,5,6,7-tetrahydro-1*H*-isoindole-1,3(2*H*)-dione in/on application verification pads.

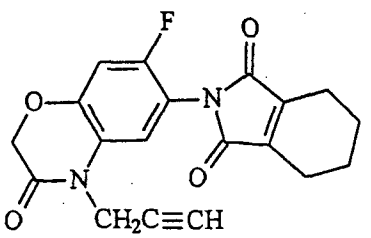
Briefly, flumioxazin residues are extracted from application verification pads using acetone. A 1.0 mL aliquot is diluted with acetone and the flumioxazin is quantitated by gas chromatography using a nitrogen-phosphorus specific flame-ionization detector (NPD).

REAGENTS

Acetone - pesticide quality or equivalent.

REFERENCE STANDARD

Flumioxazin - analytical standard of known purity.



Prepare a stock solution containing 1.0 mg/mL in acetone. This solution will also be used as fortifying solution. Prepare a minimum of four linearity standards by diluting this stock solution with acetone to concentrations ranging from 0.2 to 10 µg/mL (See Note 1). Prepare a calibrating solution containing 2.0 µg/mL by diluting the stock solution with toluene. (The calibrating solution may be used as one of the four required linearity standards). All solutions should be kept refrigerated when not in use.

EQUIPMENT

Aluminum Foil

Flasks – 25 mL volumetric.

Jars – VWR, 250 mL, tall wide mouth glass with Teflon-lined lids, part # 15900-160.

Gas chromatograph – Hewlett-Packard Model 5890, equipped with a packed column glass insert for direct injection(HP Part # 5080-8732, packed with approximately 5mm of silanized glass wool), an NP detector, automatic sampler, and integrator or equivalent system.

Reciprocating shaker – Eberbach or equivalent.

Solvent saturation Pads – Alltech, 20 x 20 cm, part # 7630

Vials – 6 dram, with polyethylene-lined screw caps or equivalent.

ANALYTICAL PROCEDURE

1. Extraction

If required by testing facility, control samples for method recovery should be fortified with flumioxazin (See Note 2). Place two solvent saturation pads side by side on a piece of aluminum foil. Fortify the two pads evenly using the fortifying solution. Flip one of the pads on top of the other pad. Fold the two solvent saturation pads together in half. Starting from the shorter end, roll the two pads together and place in a 250 mL glass jar. Discard the aluminum foil.

Add 100 mL of acetone to each sample jar. Place the jar on its side in a reciprocating shaker and shake for 10 minutes. Transfer 1.0 mL of the extract to 25 mL volumetric flask and bring up to volume with acetone. Transfer to a 6 dram screw cap vial for storage. Store at ≤ 0°C until GC analysis.

2. Gas Chromatography Measurement

Analyze a range of linearity standards with the analytical sequence. The recommended sequence of samples and standards for analysis is: calibrating standard, sample, sample, sample, calibrating standard, etc. This sequence may, however, be modified if the reproducibility requirement is met. (See Note 3). Each sequence must begin and end with a calibrating standard. The area integration results of each unknown sample must be less than the maximum value obtained during determination of instrument linearity or the sample needs to be diluted and re-injected.

Transfer a portion of the sample extract to an autosampler vial and analyze by GC/NPD, along with the calibrating and linearity standard solutions, using the following operating conditions:

Column: DB-17 (15 M x 0.53 mm i.d., 1.5 μm film) J & W Scientific Cat. # 125-1712 or equivalent.

Column temperature program:
Initial temperature: 250°C
Initial hold time: 1.0 minute
Program rate: 20°C/minute
Program final temperature: 270°C
Program hold time: 8 minutes

Injector temperature: 275°C
Detector temperature: 300°C
Carrier Gas and flow rate: Helium, 10 mL/min.
Makeup Gas and flow rate: Helium, 20 mL/min.
Injection volume: 1.0 μL

Retention time: 6.99 minutes

The GC parameters shown above are given only as a guide. They may be modified as needed to optimize the chromatography or to resolve matrix interferences. Each set of chromatograms must be clearly labeled with the GC parameters used.

If matrix interferences are encountered during the analysis of flumioxazin, sample extracts may be reinjected using the following alternate GC/NPD parameters:

Column: DB-5 (30 M x 0.53 mm i.d., 1.5 μm film) J & W Scientific Cat. # 125-5032 or equivalent.

Column temperature program:
Initial temperature: 250°C
Initial hold time: 1.0 minute
Program rate: 20°C/minute
Program final temperature: 275°C
Program hold time: 8 minutes

Injector temperature: 250°C
Detector temperature: 300°C
Carrier Gas and flow rate: Helium, 10 mL/min.

Makeup Gas and flow rate: Helium, 20 mL/min.
Injection volume: 1.0 µL

Retention time: 6.77 minutes

The instrument parameters shown above are given only as a guide. They may be modified as needed to optimize the chromatography or to resolve matrix interferences. Each set of chromatograms must be clearly labeled with the GC parameters used.

3. Calculations

The amount of flumioxazin in each sample is calculated using the following formula:

$$ug/cm^2 = \frac{B \times C \times FV \times DF}{A \times SA}$$

where:

B = Integration counts for the analyte in the sample chromatogram.
C = Concentration of analyte in the calibrating standard (2.0 µg/mL).
FV = Final volume of extract (100 mL).
DF = Dilution factor, used if sample extract is diluted prior to analysis (25).
A = Mean integration counts for the analyte in the calibrating solutions.
SA = Sample pad area in cm^2 (800 cm^2).

This can be converted to grams/acre by using the following:

$$g/acre = (ug/cm^2)(10^{-6}\, g/ug)(40.468x10^6\, cm^2/acre)$$
$$= (ug/cm^2)(40.468)$$

where:
µg/cm^2 = µg/cm^2 found in the sample.

LIMITS OF QUANTITATION AND DETECTION

The validated limit of quantitation (LOQ) of flumioxazin in/on application verification pads analyzed by this method is 1.25 ug/cm^2. The estimated limit of detection (LOD) is 0.625 ug/cm^2 when 1.0 mL of sample extract is diluted to 25 mL as specified in the method.

VALENT U.S.A. CORPORATION
Valent Technical Center
Dublin, California

DETERMINATION OF FLUMIOXAZIN, THPA, HPA, AND SAT-482-HA IN SOIL

Method: RM-30S-1 Date: November 21, 2002

I. INTRODUCTION

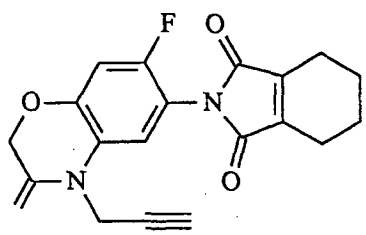
The following method describes the determination of the flumioxazin and its degradates THPA, HPA, and SAT-482-HA in soil. Briefly, the method involves extracting a 20 g soil sample with 80 mL of acetone/1% HCl (9:1, v/v) and with 80 mL acetonitrile/1% HCl (8:2, v/v). The combined extract is split - with half of the extract partitioned to allow for flumioxazin analysis by GC/MS, and a fourth of the extract cleaned up on a C_{18} cartridge for THPA, HPA, and SAT-482-HA analyses by LC/MS-MS.

The flumioxazin residues are isolated by rotary evaporating the organic solvent and then partitioning the residues from the aqueous solution into hexane. The hexane is removed by rotary evaporation, the residues are dissolved in toluene, and the concentrated extract is analyzed by GC/MS.

The THPA, HPA, and SAT-482-HA residues are isolated by rotary evaporating the organic solvent, centrifuging the sample to separate the dissolved residues (THPA and HPA), and loading the residues in the supernatant liquid onto a C_{18} cartridge. The cartridge is rinsed with water to remove acid, the THPA and HPA residues are eluted with methanol/water (3:7, v/v; 0.005 M NH_4OOCH), and eluant is analyzed for LC/MS-MS for THPA and HPA. The solids obtained from centrifuging are extracted with methanol/water (3:7, v/v), the mixture is centrifuged, and the supernatant liquid is transferred onto the C_{18} cartridge. The extraction of the solids from centrifuging is repeated with methanol/water (1:1, v/v), the mixture is centrifuged, and the supernatant liquid is transferred to the cartridge as before. The SAT-482-HA is then eluted from the column with methanol/water (1:1, v/v; 0.01 M Formate Buffer), with analysis by LC/MS-MS.

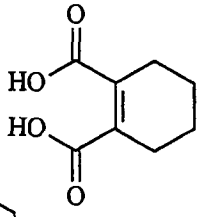
II. ANALYTICAL STANDARDS

Flumioxazin reference standard - Valent U.S.A. Corporation.

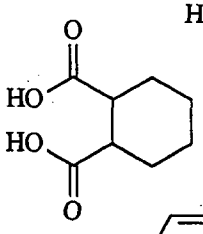


Valent U.S.A. Corporation

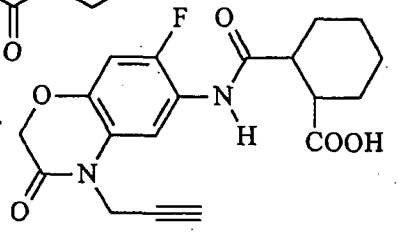
THPA reference standard - Valent U.S.A. Corporation.



HPA reference standard - Valent U.S.A. Corporation.



SAT-482-HA reference standard - Valent U.S.A. Corporation.



Flumioxazin Standard, 1.0 mg/mL Stock solution.

Weigh 0.100 grams of flumioxazin (to ensure a 1.0 mg/mL concentration, correct the amount of standard weighed for the purity of the standard) into a 100 mL volumetric flask. Dilute to volume with acetone. Store refrigerated.

THPA Standard, 1.0 mg/mL Stock solution.

Weigh 0.100 grams of THPA (to ensure a 1.0 mg/mL concentration, correct the amount of standard weighed for the purity of the standard) into a 100 mL volumetric flask. Dilute to volume with acetone/water (1:1, v/v). Store refrigerated.

HPA Standard, 1.0 mg/mL Stock solution.

Weigh 0.100 grams of HPA (to ensure a 1.0 mg/mL concentration, correct the amount of standard weighed for the purity of the standard) into a 100 mL volumetric flask. Dilute to volume with acetone. Store refrigerated.

SAT-482-HA Standard, 1.0 mg/mL Stock solution.

Weigh 0.100 grams of SAT-482-HA (to ensure a 1.0 mg/mL concentration, correct the amount of standard weighed for the purity of the standard) into a 100 mL volumetric flask. Dilute to volume with acetone. Store refrigerated.

Flumioxazin Standard, 20 µg/mL solution in acetone.

Pipette 2.0 mL of the 1.0 mg/mL Stock solution into a 100 mL volumetric flask. Dilute to volume with acetone. Store refrigerated.

Flumioxazin Standard, 20 µg/mL solution in toluene.

Pipette 2.0 mL of the 1.0 mg/mL Stock solution into a 100 mL volumetric flask. Dilute to volume with toluene. Store refrigerated.

THPA Standard, 20 µg/mL solution in methanol.

Pipette 2.0 mL of the 1.0 mg/mL Stock solution into a 100 mL volumetric flask. Dilute to volume with methanol. Store refrigerated.

HPA Standard, 20 µg/mL solution in methanol.
Pipette 2.0 mL of the 1.0 mg/mL Stock solution into a 100 mL volumetric flask. Dilute to volume with methanol. Store refrigerated.

SAT-482-HA Standard, 20 µg/mL solution in methanol.
Pipette 2.0 mL of the 1.0 mg/mL Stock solution into a 100 mL volumetric flask. Dilute to volume with methanol. Store refrigerated.

Flumioxazin Fortification Standard, 2.0 µg/mL solution in acetone.
Pipette 10.0 mL of the 20 µg/mL solution in acetone into a 100 mL volumetric flask. Dilute to volume with acetone. Store refrigerated.

LC/MS-MS Fortification Standard, 2.0 µg/mL solution in methanol.
Pipette 10.0 mL of each 20 µg/mL solution in methanol (THPA, HPA, and SAT-482-HA) into a 100 mL volumetric flask. Dilute to volume with methanol. Store refrigerated.

Flumioxazin Linearity Standard, 2.0 µg/mL solution in toluene.
Pipette 10 mL of the 20 µg/mL solution in toluene into a 100 mL volumetric flask. Dilute to volume with toluene. Store refrigerated.

Flumioxazin Linearity Standard, 1.0 µg/mL solution in toluene.
Pipette 5.0 mL of the 20 µg/mL solution in toluene into a 100 mL volumetric flask. Dilute to volume with toluene. Store refrigerated.

Flumioxazin Linearity Standard, 0.5 µg/mL solution in toluene.
Pipette 2.5 mL of the 20 µg/mL solution in toluene into a 100 mL volumetric flask. Dilute to volume with toluene. Store refrigerated.

Flumioxazin Linearity Standard, 0.2 µg/mL solution in toluene.
Pipette 1.0 mL of the 20 µg/mL solution in toluene into a 100 mL volumetric flask. Dilute to volume with toluene. Store refrigerated.

Flumioxazin Linearity Standard, 0.1 µg/mL solution in toluene.
Pipette 0.5 mL of the 20 µg/mL solution in toluene into a 100 mL volumetric flask. Dilute to volume with toluene. Store refrigerated.

THPA/HPA Linearity Standard, 2.0 µg/mL solution in methanol.
Pipette 10.0 mL of each 20 µg/mL solution in methanol (THPA and HPA) into a 100 mL volumetric flask. Dilute to volume with methanol. Store refrigerated.

THPA/HPA Linearity Standard, 1.0 µg/mL solution in methanol.
Pipette 5.0 mL of each 20 µg/mL solution in methanol (THPA and HPA) into a 100 mL volumetric flask. Dilute to volume with methanol. Store refrigerated.

THPA/HPA Linearity Standard, 0.5 µg/mL solution in methanol.

Pipette 2.5 mL of each 20 µg/mL solution in methanol (THPA and HPA) into a 100 mL volumetric flask. Dilute to volume with methanol. Store refrigerated.

THPA/HPA Linearity Standard, 0.08 µg/mL solution in methanol.

Pipette 0.4 mL of each 20 µg/mL solution in methanol (THPA and HPA) into a 100 mL volumetric flask. Dilute to volume with methanol. Store refrigerated.

SAT-482-HA Linearity Standard, 0.50 µg/mL solution in methanol.

Pipette 2.5 mL of 20 µg/mL SAT-482-HA solution in methanol into a 100 mL volumetric flask. Dilute to volume with methanol. Store refrigerated.

SAT-482-HA Linearity Standard, 0.25 µg/mL solution in methanol.

Pipette 1.25 mL of 20 µg/mL SAT-482-HA solution in methanol into a 100 mL volumetric flask. Dilute to volume with methanol. Store refrigerated.

SAT-482-HA Linearity Standard, 0.05 µg/mL solution in methanol.

Pipette 10 mL of 0.50 µg/mL SAT-482-HA solution in methanol into a 100 mL volumetric flask. Dilute to volume with methanol. Store refrigerated.

SAT-482-HA Linearity Standard, 0.025 µg/mL solution in methanol.

Pipette 5.0 mL of 0.50 µg/mL SAT-482-HA solution in methanol into a 100 mL volumetric flask. Dilute to volume with methanol. Store refrigerated.

Note: Similar dilution procedures and standard concentrations are also acceptable.

III. REAGENTS

Acetone – Pesticide quality (or equivalent)

Acetonitrile - Pesticide quality (or equivalent)

Ammonium Formate – Reagent grade

Dichloromethane - Pesticide quality (or equivalent)

Ethyl acetate - Pesticide quality (or equivalent)

Formic acid, 96% - Reagent grade

Hexane – Pesticide quality (or equivalent)

Hydrochloric Acid, 12 *N* – Analytical grade

Methanol - Pesticide quality (or equivalent)

Sodium sulfate, anhydrous – Analytical grade (Acetone washed and air dried)

Toluene - Pesticide quality (or equivalent)

Water, deionized

Water, HPLC Grade

IV. REAGENT SOLUTIONS

1% HCl solution

Dilute concentrated hydrochloric acid to obtain approximately a 1% HCl solution. For example, add 25-26 mL of concentrated HCl (12 *N*) into approximately 500 mL of deionized water, and add deionized water to set the final volume to 1 liter. Store at room temperature.
Note: Use caution when handling HCl as it is corrosive.

Acetone/1% HCl solution, 9:1 (v/v).

Combine 9 parts acetone with 1 part 1% HCl solution in a glass bottle. For example, add 900 mL of acetone and 100 mL of 1% HCl. Shake to mix. Store at room temperature.

Acetonitrile/1% HCl solution, 8:2 (v/v).

Combine 8 parts acetone with 2 parts 1% HCl solution in a glass bottle. For example, add 800 mL of acetonitrile and 200 mL of 1% HCl. Shake to mix. Store at room temperature.

Aqueous Formate Buffer. [HPLC Eluant A]

Dissolve 0.16 g ammonium formate (NH_4OOCH) and 0.098 mL formic acid (HOOCH, 96%) in 500 mL of HPLC grade water in a glass bottle. Shake to mix. Store at room temperature.

Methanol Solution, Formate Buffer. [HPLC Eluant B]

Dissolve 0.16 g ammonium formate (NH_4OOCH) and 0.098 mL formic acid (HOOCH, 96%) in 500 mL of methanol in a glass bottle. Shake to mix. Store at room temperature.

Methanol/water Solution, 1:9 (v/v). [10% MeOH]

Combine 1 part methanol with 9 parts deionized water in a glass bottle. For example, add 50 mL of methanol and 450 mL of deionized water. Shake to mix. Store at room temperature.

Methanol/water Solution, 3:7 (v/v). [30% MeOH]

Combine 3 parts methanol with 7 parts deionized water in a glass bottle. For example, add 300 mL of methanol and 700 mL of deionized water. Shake to mix. Store at room temperature.

Methanol/water Solution (3:7, v/v; 0.005 M NH_4OOCH). [30% MeOH (w/ NH_4OOCH)]
Add 0.16 g of ammonium formate (NH_4OOCH) into 500 mL of methanol/water, 3:7 (v/v) in a glass bottle. Shake well to mix. Store at room temperature.

Methanol/water Solution, 1:1 (v/v). [50% MeOH]
Combine 1 part methanol with 1 part deionized water in a glass bottle. For example, add 400 mL of methanol and 400 mL of deionized water. Shake to mix. Store at room temperature.

Methanol/water Solution, (1:1, v/v; 0.01 M Formate Buffer). [50% MeOH (w/ Buffer)]
Add 0.16 g of ammonium formate (NH_4OOCH) and 0.098 mL formic acid (HOOCH) into 500 mL of methanol/water, 1:1 (v/v) in a glass bottle. Shake well to mix. Store at room temperature.

V. EQUIPMENT

Autosampler Vials and Caps.

BAKERBOND spe Octadecyl (C_{18}) Disposable Extraction Cartridges, 6 mL Solid Phase Extraction Columns, 1000 mg per column (Cat # 7020-07)

Balances - analytical and top loading.

Büchner Funnels - 9 cm.

Centrifuge, bench-top

Centrifuge tubes – 5 mL screw-top with Teflon-lined caps (or equivalent)

Filter Flasks, Vacuum - 500 mL.

Filter Funnels - approximately 100 mm diameter.

Filter Paper, Glass Fiber - Whatman GF/A, 9 cm (or equivalent).

Glass Jars, 250 mL (or equivalent) with Teflon-lined caps

Graduated Cylinders - 1000, 500, 250, 100, and 50 mL

Heated Water Bath.

Linear Shaker, Erbach (or equivalent)

Pasteur Pipettes, Disposable – 5 3/4 and 9 inch.

Pipettes, Volumetric – 5 and 6 mL.

Pipettors, Automatic - capable of accurately dispensing volumes from 0.1 mL through 2.5 mL.

Refrigerator.

Rotary Vacuum Evaporators.

Round-bottom Flasks - 250 and 100 mL (with 24/40 ground glass joints).

Vacuum Apparatus.

Vacuum Manifold.

Vials, Screw Top – 12 mL with Polyethylene-lined caps.

Volumetric Flasks, 100 mL

VI. INSTRUMENTATION

1. GAS CHROMATOGRAPH/MASS SPECTROMETER (GC/MS)

Hewlett-Packard Model 6890 GC equipped with an HP5973 mass selective detector, an autosampler, and a ChemStation (or equivalent). Conditions shown below are suggested for this analysis (similar conditions may be used as appropriate).

Column: DB-1 (J & W Scientific, Inc.), 30 m x 0.32 mm I.D., 0.25 µm film thickness

Column Oven Temperature Program -

Initial Temperature:	200°C
Hold Time:	1.0 minute
Program Rate:	15°C/minute
Final Temperature :	320°C
Final Time:	5.0 min
Total Run Time:	14.0 min

Temperatures -

Injector:	280°C
Transfer Line:	280°C
Detector:	300°C

Flows -

Column (Helium):	1.0 mL/minute
Split Vent:	20 mL/minute

Injection Volume:	0.5 µL (Split mode, 2 mm ID Quartz liner with Quartz wool)
Acquisition Mode:	Selective Ion Monitoring [354 m/z]
Retention Time, Flumioxazin:	7.9 minutes (Figure 1)

2. LIQUID CHROMATOGRAPH/MASS SPECTROMETER (LC/MS-MS)

Hewlett-Packard Model 1100 HPLC with an Applied Biosystems API2000 mass spectrometer system (or equivalent) for analysis of THPA, HPA, and SAT-482-HA. Conditions shown below are suggested for this analysis (similar conditions may be used as appropriate).

Column: LUNA C_{18} Column (Phenomenex), 50 mm x 3 mm ID, 3 micron

Column Oven Temperature - 35°C

Column Flow: 0.3 mL/minute

MS Sample Introduction: Electrospray Ionization

THPA & HPA Analysis:

Injection Volume =	15 µL
Scan Type =	MRM
Polarity =	Negative
Dwell (msec) =	250
HPA, Q1 Mass (amu) =	170.8
HPA, Q3 Mass (amu) =	127.4
THPA, Q1 Mass (amu) =	169.0
THPA, Q3 Mass (amu) =	125.3

THPA & HPA Column Profile

Total Time (min)	% Eluant A (H_2O Buffer)	% Eluant B (MeOH Buffer)
0.0	70	30
1.0	70	30
7.0	20	80
9.0	20	80
10	70	30
15	70	30

Retention Times (Figure 5) -

THPA:	3.3 minutes
HPA:	6.2 minutes

SAT-482-HA Analysis:

Injection Volume =	15 µL
Scan Type =	MRM
Polarity =	Positive
Dwell (msec) =	500
SAT-482-HA, Q1 Mass (amu) =	375.0
SAT-482-HA, Q3 Mass (amu) =	221.0

SAT-482-HA Column Profile

Total Time (min)	% Eluant A (H_2O Buffer)	% Eluant B (MeOH Buffer)
0.0	60	40
1.0	60	40
9.0	10	90
12.0	10	90
13.0	60	40
18.0	60	40

Retention Time, SAT-482-HA: 8.2 minutes (Figure 9)

VII. ANALYTICAL PROCEDURES

1. WEIGHT SAMPLES

Weigh 20±0.1 g of soil into a tall screw-top glass jar. If required by the testing facility, control samples to be used for method recoveries may be fortified at 0.02 ppm and/or 0.1 ppm with each analyte (Note 1).

2. ACETONE/1% HCl EXTRACTION

Add 80 mL of acetone/1% HCl to the sample, swirl briefly, cap, and shake on a linear shaker for 30 minutes. Assemble a vacuum filtration apparatus with a 500 mL vacuum filter flask, a Büchner funnel, and a Whatman GF/A filter (pre-wet the filter with acetone to seat it in the funnel). Apply vacuum to the flask, decant the liquid into the funnel, and collect the filtrate in the filter flask. As some of the soil is likely to transfer onto the filter, transfer the filter back into the jar.

3. ACETONITRILE/1% HCl EXTRACTION

Add 80 mL of acetonitrile/1% HCl, cap the jar, and shake again on a linear shaker for 30 minutes. Vacuum filter the extract through a Whatman GF/A filter, combining the extracts.

Transfer the filtrate into a 250 mL graduated cylinder and add acetone to adjust the total volume to 160 mL. [The volume may be adjusted to 180 mL – the percentage removed for **Fraction A** and **Fraction B** should be 50% and 25%, respectively.] Mix the sample by transferring back into the flask. Transfer 80 mL of the sample (**Fraction A**) into a 250 mL round-bottom flask. Transfer 40 mL of the sample (**Fraction B**) into a 100 mL round-bottom flask. These extracts may be stored overnight (in a refrigerator or freezer).

4. HEXANE PARTITION FOR ***FRACTION A*** (FLUMIOXAZIN ANALYSIS)

Attach the 250 mL round-bottom flask (**Fraction A**) to a rotary vacuum evaporator equipped with a heated water bath (temperature < 40°C). Rotary evaporate the solvent to obtain an aqueous residue (approximately 15 mL).

Transfer the mixture into a 250 mL separatory funnel, rinse the flask with 50 mL of deionized water, and add the rinse to the separatory funnel. Rinse the round-bottom flask with 75 mL hexane, and add the hexane to the separatory funnel. Stopper the separatory funnel, invert and vent the funnel, and then shake the separatory funnel vigorously for 1 minute (with occasional venting). Allow the phases to separate (about 10 min), and then drain most of the aqueous layer back into the round-bottom flask (leaving about 5 mL of the aqueous layer). Swirl and briefly shake (with venting) to clarify the hexane layer. Allow any solids to settle. Drain the remaining aqueous layer (and any interface layer) into the round-bottom flask.

Drain the hexane layer through sodium sulfate (approximately 30 g, suspended on glass wool in a funnel and freshly washed with approximately 25 mL of hexane) into a clean 250 mL round-bottom flask. Rinse the sodium sulfate with 25 mL of hexane, combining the rinse with the extract.

5. ROTARY EVAPORATION OF HEXANE (FLUMIOXAZIN ANALYSIS)

Using a rotary evaporator (water bath temperature < 40°C), reduce the volume of hexane in the 250 mL round-bottom flask to approximately 30-40 mL. Transfer the residues into the 100 mL round-bottom flask, rinsing the 250 mL round-bottom flask with 10-15 mL ethyl acetate and adding the rinse to the 100 mL round-bottom flask.

Continue rotary evaporation of the sample, just to dryness. Add 1.0 mL of toluene to the flask, stopper and briefly sonicate to dissolve the residues. Transfer the residues into an autosampler vial for GC/MS analysis.

6. C_{18} CARTRIDGE CLEANUP OF ***FRACTION B*** (THPA, HPA, & SAT-482-HA ANALYSIS)

Place the flask containing **Fraction B** on the rotary evaporator (water bath temperature < 30°C), and completely remove the organic solvents in the 100 mL round-bottom flask. To ensure complete removal of the organic solvents, continue rotary evaporation of 10-15 minutes after the turbid sample has ceased bubbling. [If small amounts of organic solvent remain, it is very likely that the THPA & HPA will not be isolated on the C_{18} cartridge.]

Transfer the aqueous residue to a centrifuge tube using a Pasteur pipette. Rinse the round-bottom flask twice with 1 mL portions of deionized water, transferring each rinse into the centrifuge tube. Cap the centrifuge tube, and centrifuge the sample for 10-12 minutes. The total volume in the tube will be approximately 7-8 mL. *NOTE: Save the 100 mL round-bottom flask as it will be rinsed later with 30% MeOH during isolation of SAT-482-HA.*

Prepare C_{18} cartridges by rinsing the cartridges with approximately 5 mL of methanol, and 3-4 times with approximately 5 mL of deionized water. This process may be performed on a vacuum manifold using a slight vacuum to initiate flow. *NOTE: As the THPA and HPA are not readily retained on the C_{18} cartridge, care needs to be taken to ensure that air is not pulled onto the cartridge and that all of the methanol has been thoroughly rinsed from the cartridge.* Allowing the methanol to drain just by gravity instead of "with vacuum" (so that the frit can go dry without pulling air into the column) will improve the effectiveness of the subsequent water rinses.

Remove the rinsed C_{18} cartridge from the vacuum manifold, and suspend it over a vial or beaker (or other suitable container). Carefully withdraw the supernatant liquid in the centrifuge tube with a Pasteur pipette and transfer it into the cartridge. This loading process will require two transfers as the volume of liquid exceeds the volume of the cartridge. Be careful not disturb the pellet at the base of the centrifuge tube (this will require that a few drops of liquid are left in the tube). Patiently wait while the sample drains through the cartridge. *NOTE: If this process is accelerated, recoveries of THPA and HPA are likely to be reduced. Also, save the centrifuge tube with the pellet for subsequent isolation of SAT-482-HA.*

Once the sample is loaded onto the cartridge, rinse the cartridge sequentially with a 2-mL portion of deionized water and then with 1.5 mL of 10% MeOH. Each rinse should be added to the cartridge after the previous rinse has completely passed through the cartridge. Discard the accumulated eluant.

Place a vial beneath the cartridge, and add 6.0 mL of 30% MeOH (w/ NH_4OOCH) to the cartridge to elute the THPA and HPA. After the eluant is collected, cap the vial. When withdrawing an aliquot for analysis, mix the contents using a Pasteur pipette and then transfer an aliquot into an autosampler vial for THPA and HPA analysis by LC/MS-MS. *NOTE: Save the cartridge for isolating the SAT-482-HA residues.*

7. C_{18} CARTRIDGE CLEANUP FOR SAT-482-HA

Add 5 mL of 30% MeOH to the 100 mL round-bottom flask used for ***Fraction B***. Rinse the flask, and transfer the rinse with a Pasteur pipette into the centrifuge tube. Cap the tube, briefly shake to dislodge the pellet, and then vortex the sample for approximately 1 minute to disperse the solids. Centrifuge the sample for 10-12 minutes.

Place the C_{18} cartridge on a vacuum manifold with a vial or beaker (or suitable container) beneath the cartridge. Use a Pasteur pipette to carefully transfer the supernatant liquid (30% MeOH) in the centrifuge tube into the cartridge (don't disturb the pellet), and allow the liquid to gravity drain through the cartridge.

Add 5 mL of 50% MeOH to the centrifuge tube. Cap the tube, briefly shake to dislodge the pellet, and then vortex the sample for approximately 1 minute to disperse the solids. Centrifuge the sample for 10-12 minutes.

While the samples are centrifuging, check the flow rate through the C_{18} cartridges. A slight amount of vacuum may be applied to increase the drip rate to approximately 2 seconds between drops. Care should be taken to ensure that air is not drawn into the cartridges.

Once the 30% MeOH extract has passed through the cartridge, use a Pasteur pipette to carefully transfer the supernatant liquid (50% MeOH) in the centrifuge tube into the cartridge (as before, don't disturb the pellet). A slight amount of vacuum may again be applied to set the flow at approximately 2 seconds per drop. *Do not pull air into the cartridge.*

After the 50% MeOH has passed through the cartridge, place a vial beneath the cartridge and elute the SAT-482-HA with 10 mL (2x5 mL) of 50% MeOH (w/ Buffer). After the eluant is collected, cap the vial. Before withdrawing an aliquot for analysis, mix the contents using a Pasteur pipette.

As ion suppression has been observed for SAT-482-HA, two-fold (2x) dilution of the extract is recommended prior to analysis. Add 0.75 mL of 50% MeOH (with Formate Buffer) directly to the autosampler vial, add 0.75 mL of the sample extract, cap the vial, and analyze for SAT-482-HA by LC/MS-MS. *Note: Similar dilution procedures are also acceptable.*

8. GC/MS MEASUREMENT FOR FLUMIOXAZIN

Prepare an analytical sequence as follows: Condition the instrument with at least five injections of a sample extract. Include within the analytical sequence a range of at least four standard concentrations to establish the linear response of the GC/MS, including a 1.0 µg/mL standard. [A typical set of linearity standards would include a 0.1, 0.5, 1.0, and 2.0 µg/mL, with an injection volume of 0.5 µL and a split ratio of 20:1.] To verify the linear response, the response factor for each of the four standards is calculated [dividing the peak area of each standard by its concentration], the standard deviation of the four response factors is determined, and then the standard deviation is divided by the average response factor. The result (the coefficient of variation) must be 10% or less for the instrument response to be considered linear for that range of standards.

A sample sequence is constructed with the following order: a reference standard (1.0 µg/mL), a set of 1 to 4 sample extracts, a linearity standard, another set of 1 to 4 sample extracts, ..., and a reference standard. The sequence must begin and end with reference standards. The coefficient of variation of the reference standard responses must be 10% or less for the analysis set to be acceptable.

If the peak response for an analyte in a sample is greater than the peak response of the highest linearity standard, the sample extract must be diluted and the diluted extract analyzed. The sample extract must be diluted (with toluene) such that each peak obtained is within the documented linear response range of the GC/MS.

9. LC/MS-MS MEASUREMENT FOR THPA AND HPA

Dilute the analytical standards by adding 1.0 mL of each to a 10.0 mL volumetric flask and then setting the volume with 30% MeOH (w/ NH_4OOCH). This will result in a set of standards from 0.008 to 0.2 µg/mL of THPA and HPA. These standards may be reused on subsequent analytical runs.

Prepare an analytical sequence as follows: Condition the instrument with at least four injections of a THPA/HPA analytical standard and/or a sample extract. Include within the analytical sequence a range of at least four standard concentrations to establish the linear response of the LC/MS-MS, including a 0.1 µg/mL standard. [A typical set of linearity standards would include a 0.008, 0.05, 0.1, and 0.2 µg/mL, with an injection volume of 15 µL.] To verify the linear response, the response factor for each of the four standards is calculated [dividing the peak area of each standard by its concentration], the standard deviation of the four response factors is determined,

and then the standard deviation is divided by the average response factor. The result (the coefficient of variation) must be 10% or less for the instrument response to be considered linear for that range of standards.

A sample sequence is constructed with the following order: a reference standard (0.1 µg/mL), a set of 1 to 4 sample extracts, a linearity standard, another set of 1 to 4 sample extracts, ..., and a reference standard. The sequence must begin and end with reference standards. The coefficient of variation of the reference standard responses must be 10% or less for the analysis set to be acceptable.

If the peak response for an analyte in a sample is greater than the peak response of the highest linearity standard, the sample extract must be diluted and the diluted extract analyzed. The sample extract must be diluted [with 30% MeOH (w/ NH_4OOCH)] such that each peak obtained is within the documented linear response range of the LC/MS-MS.

10. LC/MS-MS MEASUREMENT FOR SAT-482-HA

Dilute the analytical standards by adding 1.0 mL of each to a 10.0 mL volumetric flask and then setting the volume with 50% MeOH with Formate Buffer. This will result in a set of standards from 0.0025 to 0.05 µg/mL of SAT-482-HA. These standards may be reused on subsequent analytical runs.

Prepare an analytical sequence as follows: Condition the instrument with at least four injections of a SAT-482-HA analytical standard and/or a sample extract. Include within the analytical sequence a range of at least four standard concentrations to establish the linear response of the LC/MS-MS, including a 0.025 µg/mL standard. [A typical set of linearity standards would include a 0.0025, 0.005, 0.025, and 0.05 µg/mL, with an injection volume of 15 µL.] To verify the linear response, the response factor for each of the four standards is calculated [dividing the peak area of each standard by its concentration], the standard deviation of the four response factors is determined, and then the standard deviation is divided by the average response factor. The result (the coefficient of variation) must be 10% or less for the instrument response to be considered linear for that range of standards.

A sample sequence is constructed with the following order: a reference standard (0.025 µg/mL), a set of 1 to 4 sample extracts, a linearity standard, another set of 1 to 4 sample extracts, ..., and a reference standard. The sequence must begin and end with reference standards. The coefficient of variation of the reference standard responses must be 10% or less for the analysis set to be acceptable.

If the peak response for an analyte in a sample is greater than the peak response of the highest linearity standard, the sample extract must be diluted and the diluted extract analyzed. The sample extract must be diluted (using 50% MeOH w/ Buffer) such that each peak obtained is within the documented linear response range of the LC/MS-MS.

11. CALCULATIONS

The amount of analyte in each sample is calculated as follows:

$$Sample\ concentration,\ ppm\ (\mu g/g) = \frac{AxBxCxD}{ExFxG}$$

where: A = Sample peak area (in Peak Units)
B = Reference standard concentration (e.g.- 1.0, 0.1, or 0.025 µg/mL)
C = Final extract volume (e.g.- 1.0, 6.0, or 10 mL)
D = Dilution factor, if any
E = Average reference standard peak area (in Peak Units)
F = Initial sample weight (20 g)
G = Ratio of aliquot volume/total volume (e.g.- 80mL/160mL or 40mL/160mL)

VIII. LIMIT OF DETECTION

The limit of detection (LOD) of this method is 0.01 ppm (µg/g) for each analyte; and the validated limit of quantitation (LOQ) is 0.02 ppm (µg/g).

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A GC/NPD was used for analysis of the last storage stability set for flumioxazin. The following two pages (from method RM-30S-1-1) include the parameters for setup of the GC/NPD.

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ALTERNATE COLUMN CONDITIONS-

Column: Rtx-200 (Restek), 30 m x 0.32 mm I.D., 0.5 µm film thickness

Column Oven Temperature Program -

Initial Temperature:	200°C
Hold Time:	1.0 minute
Program Rate:	20°C/minute
Final Temperature :	300°C
Final Time:	8.0 min
Total Run Time:	14.0 min

Flows -

Column (Helium):	1.2 mL/minute
Split Vent:	18 mL/minute

Retention Time, Flumioxazin: 9.6 minutes

2. GAS CHROMATOGRAPH/NITROGEN-PHOSPHOROUS DETECTOR (GC/NPD)

Hewlett-Packard Model 6890 GC equipped with a nitrogen-phosphorous detector, an on-column injector, an autosampler, and a ChemStation (or equivalent). Conditions shown below are suggested for this analysis (similar conditions may be used as appropriate).

Column: Rtx-200 (Restek), 15 m x 0.53 mm I.D., 0.5 µm film thickness

On-Colum Temperature Program -

Initial Temperature:	130°C
Hold Time:	0.1 minute
Program Rate:	1250°C/minute
Final Temperature :	300°C
Final Time:	10 min

Column Oven Temperature Program -

Initial Temperature:	125°C
Hold Time:	0 minute
Program Rate:	30°C/minute
Final Temperature :	280°C
Final Time:	13 min
Total Run Time:	18.2 min

Detector Temperature: 280°C

Flows -

Column (Helium):	2.0 mL/minute
Detector, Makeup (He):	4.0 mL/minute
Detector, Hydrogen:	3.0 mL/minute
Detector, Air:	60 mL/minute

Injection Volume: 1.5 µL (on-column injection)

Retention Time, Flumioxazin: 12.7 minutes (Figure 5)

3. LIQUID CHROMATOGRAPH/MASS SPECTROMETER (LC/MS-MS)

Hewlett-Packard Model 1100 HPLC with an Applied Biosystems API2000 mass spectrometer system (or equivalent) for analysis of THPA and HPA. Conditions shown below are suggested for this analysis (similar conditions may be used as appropriate).

Column: LUNA C_{18} Column (Phenomenex), 50 mm x 3 mm ID, 3 micron

Column Oven Temperature - 35°C

Column Flow: 0.3 mL/minute

MS Sample Introduction: Electrospray Ionization

THPA & HPA Analysis:

Injection Volume =	15 µL
Scan Type =	MRM
Polarity =	Negative
Dwell (msec) =	250
HPA, Q1 Mass (amu) =	170.8
HPA, Q3 Mass (amu) =	127.4
THPA, Q1 Mass (amu) =	169.0
THPA, Q3 Mass (amu) =	125.3

HPLC Eluant Profile

Total Time (min)	% Eluant A (H_2O Buffer)	% Eluant B (MeOH Buffer)
0.0	70	30
1.0	70	30
7.0	20	80
9.0	20	80
10	70	30
15	70	30

Retention Times (Figure 8) -

THPA:	3.3 minutes
HPA:	6.2 minutes

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Calculation of a weighted linear fit with a non-zero intercept was used throughout this study. The following four pages (from method RM-30S-1-1) describe this calculation and the requirements for acceptance of the data.

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Once the sample is loaded onto the cartridge, rinse the cartridge sequentially with a 2-mL portion of deionized water and then with 1.5 mL of 10% MeOH. Each rinse should be added to the cartridge after the previous rinse has completely passed through the cartridge. Discard the accumulated eluant.

Place a vial beneath the cartridge, and add 6.0 mL of 30% MeOH (w/ NH_4OOCH) to the cartridge to elute the THPA and HPA. After the eluant is collected, cap the vial. When withdrawing an aliquot for analysis, mix the contents using a Pasteur pipette and then transfer an aliquot into an autosampler vial for THPA and HPA analysis by LC/MS-MS. Store the sample extracts in the freezer (or refrigerator) prior to analysis – *it may be necessary to analyze only a single analytical set at one time as apparent loss of THPA has been observed in some sample extracts left at room temperature.*

7. GC/MS MEASUREMENT FOR FLUMIOXAZIN

Prepare an analytical sequence as follows: Condition the instrument with at least five injections of a sample extract. Include within the analytical sequence a range of at least four standard concentrations to establish the linear response of the GC/MS, including a 1.0 µg/mL standard *in toluene*. [A typical set of linearity standards would include a 0.1, 0.5, 1.0, and 2.0 µg/mL, with an injection volume of 0.5 µL and a split ratio of 20:1.] To verify the linear response, a weighted linear fit (1/concentration) is performed for concentration versus Peak Units (typically, Area/1000). The r^2 value must be greater than 0.99 for the calibration to be acceptable. In addition, the concentration for each of the standards is recalculated using the linear fit, and the calculated concentrations must be within 10% of the theoretical concentration (except for 15% for the lowest standard) for the calibration to be acceptable.

An analytical sequence is typically constructed with the following order: conditioning sample extracts, a reference standard (1.0 µg/mL), a set of 1 to 4 sample extracts, a linearity standard, another set of 1 to 4 sample extracts, ..., and a reference standard. The sequence must begin and end with a reference standard, with at least three reference standards analyzed in the sequence. The coefficient of variation of the reference standard responses must be 15% or less for the analysis set to be acceptable.

If the peak response for an analyte in a sample extract is greater than the peak response of the highest linearity standard, the sample extract must be diluted and the diluted extract analyzed. The sample extract must be diluted (with toluene) such that each peak obtained is within the documented linear response range of the GC/MS.

8. GC/NPD MEASUREMENT FOR FLUMIOXAZIN (ALTERNATE ANALYSIS)

Prepare an analytical sequence as follows: Condition the instrument with at least five injections of a sample extract. Include within the analytical sequence a range of at least four standard concentrations to establish the linear response of the GC/NPD, including a 0.5 µg/mL standard *in acetone*. [A typical set of linearity standards would include a 0.05, 0.1, 0.5, and 1.0 µg/mL, with an injection volume of 1.5 µL.] To verify the linear response, a weighted linear fit (1/concentration) is performed for concentration versus Peak Units (typically, Area/1000). The r^2 value must be greater than 0.99 for the calibration to be acceptable. In addition, the concentration

for each of the standards is recalculated using the linear fit, and the calculated concentrations must be within 10% of the theoretical concentration concentration (except for 15% for the lowest standard) for the calibration to be acceptable.

An analytical sequence is typically constructed with the following order: conditioning sample extracts, a reference standard (0.5 µg/mL), a set of 1 to 4 sample extracts, a linearity standard, another set of 1 to 4 sample extracts, ..., and a reference standard. The sequence must begin and end with a reference standard, with at least three reference standards analyzed in the sequence. The coefficient of variation of the reference standard responses must be 10% or less for the analysis set to be acceptable.

If the peak response for an analyte in a sample extract is greater than the peak response of the highest linearity standard, the sample extract must be diluted and the diluted extract analyzed. The sample extract must be diluted (with acetone) such that each peak obtained is within the documented linear response range of the GC/NPD.

9. LC/MS-MS MEASUREMENT FOR THPA AND HPA

Dilute each of the standards (in methanol) by adding 1.0 mL to a 10.0 mL volumetric flask and then setting the volume to 10 mL with 30% MeOH (w/ NH_4OOCH). This will result in a set of standards ranging from 0.008 to 0.2 µg/mL of THPA and HPA. These standards may be refrigerated and reused on subsequent analytical runs. [As only small volumes are prepared, the diluted standards should be discarded after two weeks.]

Prepare an analytical sequence as follows: Condition the instrument with at least four injections of a THPA/HPA analytical standard and/or a sample extract. Include within the analytical sequence a range of at least four standard concentrations to establish the linear response of the LC/MS-MS, including a 0.1 µg/mL standard. [A typical set of linearity standards would include a 0.008, 0.05, 0.1, and 0.2 µg/mL, with an injection volume of 15 µL.] To verify the linear response, a weighted linear fit (1/concentration) is performed for concentration versus Peak Units (typically, Area/1000). The r^2 value must be greater than 0.99 for the calibration to be acceptable. In addition, the concentration for each of the standards is recalculated using the linear fit, and the calculated concentrations must be within 10% of the theoretical concentration (except for 15% for the lowest standard) for the calibration to be acceptable.

A sample sequence is typically constructed with the following order: conditioning sample extracts, a reference standard (0.1 µg/mL), a set of 1 to 4 sample extracts, a linearity standard, another set of 1 to 4 sample extracts, ..., and a reference standard. The sequence must begin and end with a reference standard, with at least three reference standards analyzed in the sequence. The coefficient of variation of the reference standard responses must be 15% or less for the analysis set to be acceptable.

If the peak response for an analyte in a sample extract is greater than the peak response of the highest linearity standard, the sample extract must be diluted and the diluted extract analyzed. The sample extract must be diluted [with 30% MeOH (w/ NH_4OOCH)] such that each peak obtained is within the documented linear response range of the LC/MS-MS.

10. CALCULATIONS

The amount of analyte in each sample is calculated using a weighted linear regression of concentration versus Peak Units (typically, Area/1000). The weighting (1/concentration) was done by replicating the entries in the data set prior to performing a linear regression in Excel. Examples of typical standard sets with the number of entries (to provide weighting relative to the highest standard concentration) are shown below:

For Flumioxazin by GC/MS:

Standard	Number of Entries in Data Set
2.0 ppm	1
1.0 ppm	2
0.5 ppm	4
0.1 ppm	20

For Flumioxazin by GC/NPD:

Standard	Number of Entries in Data Set
1.0 ppm	1
0.5 ppm	2
0.1 ppm	10
0.05 ppm	20

For THPA & HPA by LC/MS-MS:

Standard	Number of Entries in Data Set
0.2 ppm	1
0.1 ppm	2
0.05 ppm	4
0.008 ppm	25

The analyte concentration in each final extract is calculated from the weighted linear fit:

Analyte Extract Concentration, µg/mL = [slope x (Analyte Response, Peak Units)] + intercept

Residues in the samples are then calculated from the concentrations found in the extracts (using the equivalent sample weight resulting from the split of the initial extraction during sample preparation):

$$\text{Residues, ppm} = \frac{\textit{Extract Concentration, } \mu\text{g/mL} \times \textit{Final Volume, } \text{mL} \times \textit{Dilution Factor}}{\textit{Equivalent Sample Weight, } \text{g}}$$

For example, flumioxazin residue concentrations (when analyzed by GC/MS) would be calculated as

$$\text{Flumioxazin, ppm} = \frac{\textit{Extract Concn, } \mu\text{g/mL} \times 1.0\text{ mL} \times \textit{Dilution Factor}}{10\text{ g}}$$

For example, flumioxazin residue concentrations (when analyzed by GC/NPD) would be calculated as

$$\text{Flumioxazin, ppm} = \frac{\textit{Extract Concn, } \mu\text{g/mL} \times 2.0\text{ mL} \times \textit{Dilution Factor}}{10\text{ g}}$$

For example, THPA or HPA residue concentrations (analyzed by LC/MS-MS) would be calculated as

$$\text{THPA or HPA, ppm} = \frac{\textit{Extract Concn, } \mu\text{g/mL} \times 6.0\text{ mL} \times \textit{Dilution Factor}}{5\text{ g}}$$

Fortified sample percentage recoveries are calculated by (1) subtracting the area in the control sample from the area in the fortified sample, (2) calculating the corrected analyte concentration, and then (3) calculating percent recovery:

Corrected Response [units as Area/1000] = Fortified Sample Response – Control Sample Response

Corrected Analyte Concentration, ppm =

$$\frac{[\text{slope} \times (\textit{Corrected Response}) + \text{intercept}],\ \mu\text{g/mL} \times \textit{Final Volume, mL} \times \textit{Dilution Factor}}{\textit{Equivalent Sample Weight, g}}$$

$$\text{Recovery, \%} = \frac{\textit{Corrected Analyte Concentration, ppm}}{\textit{Theoretical Analyte Concentration, ppm}} \times 100\%$$

VIII. LIMIT OF DETECTION

The limit of detection (LOD) of this method is 0.01 ppm (µg/g) for each analyte; and the validated limit of quantitation (LOQ) is 0.02 ppm (µg/g).