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DETERMINATION OF FLUMIOXAZIN, THPA, AND HPA IN SOIL

Method: RM-30S-1-1 Date: September 30, 2004

I. INTRODUCTION

The following method describes the determination of the flumioxazin and its degradates, THPA and HPA, 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 (or GC/NPD), and with a fourth of the extract cleaned up on a C_{18} cartridge for THPA and HPA analysis by LC/MS-MS.

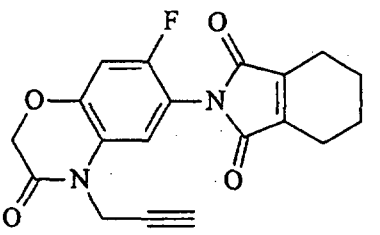
The flumioxazin residues are isolated by rotary evaporating the organic solvent and then partitioning the residues from the aqueous solution into hexane. The hexane is removed by rotary evaporation, the residues are dissolved in toluene, and the concentrated extract is analyzed by GC/MS or GC/NPD.

The THPA and HPA residues are isolated by rotary evaporating the organic solvent, centrifuging the sample to separate the dissolved THPA and HPA residues, 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 the eluant is analyzed by LC/MS-MS.

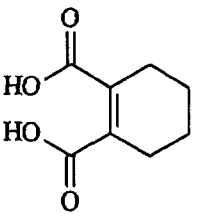
This method is based on RM-30S-1. Revisions include removing analysis for SAT-482-HA (not observed in field dissipation studies), and specifying conditions for flumioxazin analysis by GC/NPD and for instrument calibration using a weighted linear fit from the standard responses.

II. ANALYTICAL STANDARDS

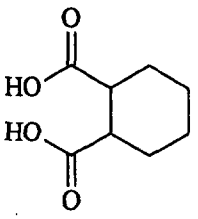
Flumioxazin reference standard - Valent U.S.A. Corporation.



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



HPA 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. [*See Note 1*]

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. [*See Note 1*]

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. [*See Note 1*]

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.

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. [*See Note 1*]

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 and HPA) into a 100 mL volumetric flask. Dilute to volume with methanol. Store refrigerated. [*See Note 1*]

Flumioxazin Linearity Standard, 2.0 µg/mL solution in toluene (for GC/MS analysis).
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 (for GC/MS analysis).
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. [*See Note 2*]

Flumioxazin Linearity Standard, 0.5 µg/mL solution in toluene (for GC/MS analysis).
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, 0.1 µg/mL solution in toluene (for GC/MS analysis).
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.

Flumioxazin Linearity Standard, 1.0 µg/mL solution in acetone (*for GC/NPD analysis*).
Pipette 5.0 mL of the 20 µg/mL solution in acetone into a 100 mL volumetric flask. Dilute to volume with acetone. Store refrigerated.

Flumioxazin Linearity Standard, 0.5 µg/mL solution in acetone (*for GC/NPD analysis*).
Pipette 2.5 mL of the 20 µg/mL solution in acetone into a 100 mL volumetric flask. Dilute to volume with acetone. Store refrigerated. [*See Note 2*]

Flumioxazin Linearity Standard, 0.1 µg/mL solution in acetone (*for GC/NPD analysis*).
Pipette 1.0 mL of the 20 µg/mL solution in acetone into a 100 mL volumetric flask. Dilute to volume with acetone. Store refrigerated.

Flumioxazin Linearity Standard, 0.05 µg/mL solution in acetone (*for GC/NPD analysis*).
Pipette 0.5 mL of the 20 µg/mL solution in acetone into a 100 mL volumetric flask. Dilute to volume with acetone. 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. [*See Notes 2 and 3*]

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.

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 acetonitrile 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.

Note: Similar dilution procedures to those shown above are also acceptable.

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)

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

VII. ANALYTICAL PROCEDURES

1. WEIGH 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 (typically, at 0.02 ppm and/or 0.1 ppm) with each analyte [*see Note 4*].

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 (or 250 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. If analyzing by GC/MS, add 1.0 mL of toluene to the flask, stopper and briefly sonicate to dissolve the residues. *If analyzing by GC/NPD, add 2.0 mL of acetone to the flask, stopper and briefly sonicate to dissolve the residues.* Transfer the residues into an autosampler vial for GC analysis.

6. C_{18} CARTRIDGE CLEANUP OF ***FRACTION B*** (THPA & HPA 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 (or 250 mL) round-bottom flask. To ensure complete removal of the organic solvents, continued rotary evaporation may be required for 10-15 minutes after the turbid sample has ceased bubbling – the final volume of aqueous residue should be <5 mL (the final volume may be 2-3 mL). *If small amounts of organic solvent remain, it is very likely that the THPA & HPA will not be isolated on the C_{18} cartridge.*

Briefly swirl and/or sonicate the sample to dislodge residues on the flask, and transfer the aqueous residue to a centrifuge tube using a Pasteur pipette. Rinse the round-bottom flask twice with 1-mL to 2-mL portions of deionized water, transferring each rinse into the centrifuge tube. The total volume in the tube should be approximately 6-7 mL. [Although the sample volume in the centrifuge tube may be adjusted with additional deionized water, *the capacity of the centrifuge tube is only slightly more than 7 mL.*] Cap the centrifuge tube, and centrifuge the sample for 10-12 minutes.

Prepare C_{18} cartridges by rinsing the cartridges with approximately 5 mL of methanol, and 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 <u>all</u> 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.

If a vacuum manifold is used, ensure that there is no vacuum (or remove the C_{18} cartridges from the vacuum manifold, suspending them over vials or other suitable containers). For each sample, carefully withdraw the supernatant liquid in the centrifuge tube with a Pasteur pipette and transfer it into the cartridge. This loading process may 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.*

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).