

## Flufenacet

Bayer Corporation

106406-1

### 3.0 Experimental

#### 3.0.1 Location

This study was conducted between May, 1993 and May, 1995 at the Bayer Research Park near Stilwell, KS. Raw data and the final report are archived at Bayer, Kansas City, MO.

### 3.1 Materials

#### 3.1.1 Apparatus

Assorted clamps and clamp holders.

Assorted laboratory glassware (including, but not limited to)

- 470 mm Allihn condenser with 24/40 ground glass joints (Kontes Glass Co., Vineland, NJ, #431000-2430 or equivalent).
- 1000-ml flat bottom flask with a 24/40 ground glass joint.
- 500-ml flat bottom flask with a 24/40 ground glass joint.
- 13-ml graduated centrifuge tubes (Kontes #410550-0013 or equivalent).
- 5-ml graduated centrifuge tube (Kontes #410550-0005 or equivalent).
- 500-ml separatory funnel.
- Short path distillation head with 24/40 ground glass joints (Kontes #513750-0000 or equivalent) and a ground glass stopper to fit the top of the distillation head and Teflon sleeves to fit 24/40 ground glass joints (Aldrich Chemical Company, Inc. Milwaukee, WI, #Z10,488-4 or equivalent).
- 35 to 50 mm Teflon jacketed magnetic stirring bar.
- Volumetric pipets and flasks.

Autosampler vials and septa (to fit the autosampler of the gc/ms).

Gas chromatograph/mass spectrometer (Hewlett Packard Company, Wilmington, DE, model HP 5890, HP 5995, or equivalent) capable of capillary column chromatography and equipped with an autosampler, a mass selective detector with appropriate data collection hardware and software, and a fused silica capillary column: 0.20 mm i.d. x 12 m, methyl silicone, 0.33  $\mu$ m film thickness (Hewlett Packard, Ultra-1 or equivalent).

Gastight microliter syringes, 100  $\mu$ l, 250  $\mu$ l, and 500  $\mu$ l (Hamilton, Inc. Reno, NV, #1700 or equivalent).

Ice bucket, about 4 liter capacity.

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N-EVAP analytical evaporator (Organomation Associates Inc., South Berlin, MA, Model N-EVAP or equivalent).

Stirrer/hot plate (Corning Inc., Corning, NY, model PC-351, PC-320, or equivalent).

Vacuum manifold for processing solid phase extraction cartridges (J. T. Baker Inc., Philipsburg, NJ, Baker spe-12 or equivalent).

Waring Laboratory Blendor and 1-liter Blendor jar (Waring Products Division, New York, NY, model 700G or equivalent).

### 3.1.2 Reagents/Supplies

Antifoam A (Dow Corning, Midland, MI) or equivalent (ie. Antifoam A Concentrate, Sigma #A 5633).

Crushed ice.

Deionized water.

4-Dimethylaminopyridine (DMAP), 99+% (Aldrich, #33,245-3 or equivalent).

Glass wool.

Granular anhydrous sodium sulfate (Mallinckrodt Speciality Chemicals Co., Paris, KN, AR grade, #8024 or equivalent).

Hydrochloric acid, 37% aqueous solution (Mallinckrodt, AR grade, #2062, or equivalent).

Monoperoxyphthalic acid, magnesium salt (MMPP, technical grade, Aldrich #28,320-7 or equivalent).

Octadecyl solid phase extraction cartridges, 3.0 ml volume, 0.50 g resin capacity (C-18 spe) (J. T. Baker, #7020-03 or equivalent).

Potassium permanganate, A.C.S. reagent grade (J. T. Baker #3227-01 or equivalent).

Pyridine, A.C.S. reagent grade (Aldrich #36,057-0 or equivalent).

Sodium bisulfite, A.C.S. reagent grade (Mallinckrodt, AR grade, #7448 or equivalent).

Sodium hydroxide, 50% (w/w) aqueous solution (Fisher Scientific, Fair Lawn, NJ, #SS254-1 or solution equivalent to 19M).

Solvents: methylene chloride, dimethylformamide, methyl *tert*-butyl ether, and acetonitrile (Burdick and Jackson Division, Baxter Healthcare Corporation, Muskegon, MI, pesticide grade).

96% Sulfuric acid, (Mallinckrodt, AR grade, #2468 or equivalent)

Sulfuric acid, 1 *N* solution in water.

Trifluoroacetic anhydride (TFAA), 99.9% (Aldrich, #10,623-2 or equivalent). Caution: This reagent is toxic and very hygroscopic. Buy the reagent in small quantities and use within 2 months of opening the bottle. Handle with care in an adequate fume hood to protect the analyst.

### 3.1.3 Standards Required

The analytical standard 4-fluoro-*N*-methylethyl benzenamine trifluoroacetamide (mw=249) may be obtained from Bayer Corporation, Agriculture Division, Environmental Research, Bayer Research Park, 17745 S. Metcalf Ave., Stilwell, KS 66085. Alternatively, the standard may be prepared as outlined in [Appendix 2](#). Analytical standards of FOE 5043 (mw=363), FOE oxalate (mw=225), FOE sulfonic acid, sodium salt monohydrate (mw=315), and FOE thioglycolate sulfoxide (mw=301) may be obtained from Bayer.

#### 3.1.3.1 Trifluoroacetamide Derivative Standards

**Primary Standard:** Using a balance accurate to 0.1 mg, weigh 0.0171 g of 4-fluoro-*N*-methylethyl benzenamine trifluoroacetamide into a 100-ml volumetric flask. Dilute the chemical to volume with methyl *tert*-butyl ether. This solution is equivalent to 250 ppm of FOE 5043 in a 10 gram sample after processing through the method.

**Secondary Standards:** Prepare additional solutions from the primary standard as follows:

(A) 25.0 ppm      Pipet 10.0 ml of the primary standard into a 100-ml volumetric flask, and dilute the solution to volume with methyl *tert*-butyl ether.

(B) 2.50 ppm      Pipet 1.00 ml of the primary standard into a 100-ml volumetric flask, and dilute the solution to volume with methyl *tert*-butyl ether.

(C) 1.00 ppm	Pipet 4.00 ml of the 25.0 ppm secondary standard into a 100-ml volumetric flask, and dilute the solution to volume with methyl <i>tert</i> -butyl ether.
(D) 0.50 ppm	Pipet 2.00 ml of the 25.0 ppm secondary standard into a 100-ml volumetric flask, and dilute the solution to volume with methyl <i>tert</i> -butyl ether.
(E) 0.25 ppm	Pipet 1.00 ml of the 25.0 ppm secondary standard into a 100-ml volumetric flask, and dilute the solution to volume with methyl <i>tert</i> -butyl ether.
(F) 0.10 ppm	Pipet 0.400 ml of the 25.0 ppm secondary standard into a 100-ml volumetric flask, and dilute the solution to volume with methyl <i>tert</i> -butyl ether.
(G) 0.05 ppm	Pipet 2.00 ml of the 2.50 ppm secondary standard into a 100-ml volumetric flask, and dilute the solution to volume with methyl <i>tert</i> -butyl ether.
(H) 0.025 ppm	Pipet 1.00 ml of the 25.0 ppm secondary standard into a 100-ml volumetric flask, and dilute the solution to volume with methyl <i>tert</i> -butyl ether.

Store the standards under refrigerated conditions ( $0\pm 3^{\circ}\text{C}$ ); under these conditions the standard solutions are stable for at least 3 months.

### 3.1.3.2 Method Validation Standards

**Primary Standards:** Using a balance accurate to 0.1 mg, weigh 0.0100 g of FOE 5043 into a 10-ml volumetric flask. Dilute the chemical to volume with methanol.

**Secondary Standards:** Prepare a 5.00  $\mu\text{g}/\text{ml}$  solution from the primary standard by pipeting 0.50 ml of primary standard into a 100-ml volumetric flask. Dilute the solution to volume with methanol.

Standard solutions of FOE oxalate; FOE sulfonic acid, sodium salt, monohydrate; and FOE thioglycolate sulfoxide should be prepared in the same manner.

Store the standards under refrigerated conditions ( $0\pm 3^{\circ}\text{C}$ ); under these conditions, the standard solutions are stable for at least 3 months.

## 3.2 Analytical Method

### 3.2.1 General Instructions

#### 3.2.1.1 Evaporations

All evaporations are done with an N-EVAP using a gentle stream of nitrogen and a room temperature ( $20\pm 5^{\circ}\text{C}$ ) water bath. Remove the sample immediately after the solvent has evaporated.

#### 3.2.1.2 Measurements

Unless otherwise indicated, all volumes should be measured with a graduated cylinder or a pipet, whichever is more convenient. Do not use micropipetors (ie. Rainen Pipetman). Unless otherwise indicated, weights should be measured on a balance capable of accuracy to 10 mg.

### 3.2.2 Detailed Procedure

#### 3.2.2.1 Sample Preparation

##### 3.2.2.1.1 Corn Forage, Corn Fodder, Corn Grain, Soybean Forage, Spinach Tops, Wheat Grain, or Wheat Straw

1. Add approximately 100 g of dry ice to a Waring Blendor jar. Add about an equal portion (100 g) of the raw agricultural commodity (RAC) to the jar in small portions. Blend the contents of the jar after each addition until a homogeneous mixture is obtained.
2. Pour the contents of the jar into doubled plastic bags, and store the open bags at  $-20\pm 3^{\circ}\text{C}$  until the last traces of dry ice have sublimed.
3. Seal and label the bags appropriately.

4. Maintain the homogenized RAC under freezer conditions,  $-20\pm 3^{\circ}\text{C}$ .

#### 3.2.2.1.2 Peanut Nutmeat, Soybean Seed, Sunflower Seed, or Turnip Roots

1. Add approximately 100 g of the RAC to a Waring Blendor jar, and blend the contents until a homogeneous mixture is obtained.
2. Pour or scrape the contents of the jar into doubled plastic bags. Seal and label the bags appropriately.
3. Maintain the homogenized RAC under freezer conditions,  $-20\pm 3^{\circ}\text{C}$ .

#### 3.2.2.1.3 Corn Oil, Soapstock, or other Processed Commodities

1. Maintain the processed RAC under freezer conditions,  $-20\pm 3^{\circ}\text{C}$ .

#### 3.2.2.2 Extraction

1. Weigh 10.0 g of the frozen sample from [3.2.2.1](#) into a 1000-ml flat bottomed boiling flask. Use a balance accurate to 0.01 g.

Note: Begin recovery samples at this point. Fortify control tissue matrix samples as described in [3.3](#).

2. Add a magnetic stirring bar and 75 ml of water to the flask. Stir the mixture for 1 hour.
3. Add 10 ml of 1.0 N sulfuric acid solution, and stir the mixture well for 1 to 2 min.
- 4a. For low moisture matrices, except corn oil, add 2.0 g of potassium permanganate to the flask, and mix the contents well, such that the entire mixture takes on a purple color from the permanganate. Stir the mixture for 5 min.
- 4b. For high moisture matrices (ie. soybean forage), add 1.0 g of potassium permanganate to the flask, and mix the contents well, such that the entire mixture takes on a purple color from the permanganate. Stir the mixture for 5 min.

- 4c. For corn oil, add 2.5 g of monoperoxyphthalic acid, magnesium salt to the flask, and mix the contents well. Stir the mixture 30 min.
5. Add 2.0 g of sodium bisulfite. Stir and swirl the flask to dissolve the bisulfite. Permanganate treated mixtures will lose any remaining purple color.
6. Very carefully pour 50 ml of concentrated sulfuric acid into the flask with the matrix mixture. Attach an Allihn reflux condenser to the boiling flask. Heat the mixture to reflux with stirring (bring to reflux within about 20 to 30 min) on a stirring hot plate.

Note: Maintain adequate water flow in the condenser to ensure complete condensation of the refluxing vapors. As the reflux will be conducted overnight, be sure water lines are firmly attached and wired to both the condenser and the water tap to prevent leaks.

7. Continue refluxing the sample for 24 hours.
8. Remove the sample from the heat, and allow the mixture to cool for about 10 to 15 min. Carefully add 350 ml of deionized water to the flask through the condenser, and then remove the condenser from the flask.
9. Cool the flask in an ice bucket half full of a crushed ice/water slurry for 15 min.
10. Slowly add (in 10 ml portions) 100 ml of 50% (w/w) sodium hydroxide solution to the flask. Stir the mixture throughout the addition. Allow time (3 to 4 min) between additions for the mixture to cool.

Note: The 50% (w/w) NaOH solution was approximately 19 M. If a more dilute solution is used, calculate the appropriate volume of solution.

11. Check the pH of the mixture with pH paper. If the pH is <12, add an additional 5 ml of 50% sodium hydroxide solution to the mixture. Recheck the pH, and, if necessary, add additional 50% sodium hydroxide to achieve a pH  $\geq$  12.
12. Add 0.5 ml of antifoaming compound to the flask. Remove the flask from the ice bath. Firmly clamp the flask on a hot plate.

Notes: If necessary, additional antifoam may be added to samples that exhibit excessive foaming during the steam distillation. At the later stages of steam distillation, the contents of the flask may boil unevenly causing the flask to “hop” or “jump” on the hot plate. Be sure the flask is firmly secured.

13. Attach a short path distillation head to the 1000-ml boiling flask containing the alkaline sample.
14. Add 2.0 ml of 37% hydrochloric acid solution to a 500-ml boiling flask. Attach the 500-ml boiling flask to the distillation head as a distillation receiver. [Figure 2](#) illustrates the distillation apparatus.

Note: Wrap the necks of the flask and the short path distillation head with aluminum foil to increase the rate of distillation.

15. Heat the contents of the 1000-ml boiling flask, with stirring, until distillation begins (within 45 to 60 min).

Note: Maintain adequate water flow in the distillation head to ensure complete condensation of the distillate. Incomplete condensation may lead to poor recoveries.

16. Distill the mixture at a rate of about 2.0 ml of distillate/min for 2.0 to 2.5 hours (about 250 to 300 ml of distillate should be collected).
17. Remove the heat source, and allow the apparatus to cool for about 10 to 15 min. Remove the 500-ml receiver flask.

Note: Allow the distillation flask to cool completely before disassembling the remainder of the distillation apparatus. If necessary, the distillate can be stored overnight before partitioning. See [3.2.2.6](#) for disposal recommendations.

### 3.2.2.3 Partitioning

Note: Perform the entire partitioning procedure without interruptions.

1. Transfer the distillate from the receiver flask, 3.2.2.2 step 17, to a 500-ml separatory funnel. Rinse the receiver flask with two, 10-ml portions of water, and add the rinses to the separatory funnel.

2. Check the pH of the distillate with pH paper. The solution should be pH 2 or less. If a pH >2 is indicated, add 1.0 ml of 37% hydrochloric acid to the solution. Recheck the pH, and if necessary add additional 37% hydrochloric acid to the solution to achieve pH #2.
3. Add 10 ml of methylene chloride to the separatory funnel, and shake the funnel for 30 sec. Allow the two phases to separate. Drain off and discard the bottom phase (methylene chloride).
4. Repeat step 3 once with a fresh 10-ml portion of methylene chloride.
5. Add 2.5 ml of 50% (w/w) sodium hydroxide solution to the separatory funnel. Check the pH of the solution in the funnel with pH paper. The pH should be 10 or greater. If a pH of <10 is indicated, add 1.0 ml of 50% sodium hydroxide solution to the funnel, and recheck the pH. If necessary, add additional 50% sodium hydroxide to achieve pH \$10.
6. Add 5.0 ml of methylene chloride to the separatory funnel, and shake the funnel for 30 sec. Allow the two phases to separate. Drain off the bottom layer (methylene chloride) into a 13-ml graduated centrifuge tube or a 20-ml glass vial.

Note: This 5 ml of extract contains the majority of the analyte. Loss of a small portion of the extract will significantly affect the recovery. Use care in handling the extract.

7. Repeat step 6 two times with 2.0-ml portions of fresh methylene chloride. Combine all the methylene chloride extracts. After the second 2 ml extraction, discard the aqueous phase.
8. Transfer the methylene chloride solution from the centrifuge tube or vial to a drying tube with a disposable Pasteur pipet (a diagram of the drying tube is shown in [Figure 3](#)). Try not to transfer any water into the drying tube. Allow the methylene chloride solution to percolate through the drying tube and into a 13-ml graduated centrifuge tube.
9. Rinse the centrifuge tube or vial two times with 0.50-ml portions of fresh methylene chloride, and transfer the rinses into the drying tube. Allow the rinses to percolate through the tube and into the 13-ml centrifuge tube. Expel the last traces of methylene chloride into the 13-ml centrifuge tube by using pressure from a pipet bulb. Rinse the drying tube with 0.50 ml of fresh

methylene chloride, and once again expel the last of the solvent from the drying tube into the 13-ml centrifuge tube.

10. Dilute the solution in the 13-ml centrifuge tube to 10.0 ml with methylene chloride.

Note: If necessary, the solution can be stored in the refrigerator ( $0\pm 5^{\circ}\text{C}$ ) before derivatization.

#### 3.2.2.4 Derivatization with Trifluoroacetic Anhydride

Note: Once begun, complete the entire derivatization process without delay.

1. Transfer a 5.0-ml aliquot of the methylene chloride solution from 3.2.2.3 step 10 to a 13-ml graduated centrifuge tube. Using gas tight syringes, add 10.0  $\mu\text{l}$  of concentrated sulfuric acid and 250  $\mu\text{l}$  of dimethylformamide (DMF) to the centrifuge tube. Mix the contents of the tube well. Evaporate the methylene chloride solvent under a gentle stream of nitrogen gas until the sample volume is  $<200\ \mu\text{l}$ . Add DMF to the sample to bring the total volume to 300  $\mu\text{l}$ .

Note: Be sure the methylene chloride solvent is completely evaporated. Residual methylene chloride may cause salt formation and possibly incomplete derivatization.

2. Place the centrifuge tube in a beaker of water at room temperature.
3. Add 100  $\mu\text{l}$  of a solution of 0.2% (w/v) DMAP in pyridine to the tube with a gas tight syringe. Cap and remove the tube from the beaker of water. Mix the contents very thoroughly. Rinse the solution up the sides of the centrifuge tube. Replace the tube into the beaker of water.

Note: Prepare the 0.2% DMAP in pyridine by adding 0.050 g of DMAP to a 25-ml volumetric flask. Dissolve the DMAP in pyridine and dilute to 25 ml. Handle the pyridine solution only in an adequate fume hood. Occasionally, a precipitate forms at the addition of the pyridine mixture to the sample. The precipitate should dissolve on addition of the TFAA in the next step. If not, the sample should be repeated with the other 5.0 ml of methylene chloride solution.

4. Slowly add 300  $\mu$ l of TFAA dropwise, using a gas tight syringe, to the centrifuge tube with gentle agitation. Cap and remove the tube from the beaker of water. Mix the contents well. Rinse the solution up the sides of the centrifuge tube. A clear yellow solution should be obtained.

Note: Handle the TFAA only in an adequate fume hood. This is a hazardous reagent; handle with care! After addition of the TFAA, the reaction mixture may form a small amount of precipitate on standing. This should not affect the results.

5. Replace the tube into the beaker of water. Allow the mixture to stand for about 15 min.
6. Mount a 3-ml, C-18 spe cartridge onto a vacuum manifold. Wash the cartridge twice with 2.5-ml portions of methyl *tert*-butyl ether and twice with 2.5-ml portions of acetonitrile, and dispose of the combined eluates. Wash the cartridge twice with 2.5-ml portions of water. Elute each solvent until the liquid level reaches the top of the sorbent bed before adding the next wash solvent. (The solvents should be eluted at a vacuum of about -2 kPa/-1.5 inches Hg) Discard the wash solvents after elution.
7. Carefully add 3.0 ml of deionized water dropwise to the centrifuge tube with gentle shaking. Dilute the solution with deionized water to 8.0 ml total volume. Cap and remove the tube from the beaker of water; mix the contents well.
8. Add the solution from step 7 to the C-18 spe cartridge in several portions. Pull the solution through the cartridge by applying a gentle vacuum (about -2 kPa/-1.5 inches Hg) to the manifold. Rinse the centrifuge tube twice with 1.0-ml portions of water, and add the rinses to the cartridge.
9. Rinse the cartridge twice with 2.5-ml portions of water. Discard the combined water eluates from the cartridge.
10. Dry the cartridge by using the vacuum manifold to draw air through the sorbent bed for 1 to 2 min (about -25 kPa/-7 inches Hg vacuum).
11. Elute the cartridge twice with 2.0-ml portions of methyl *tert*-butyl ether. The methyl *tert*-butyl ether should be pushed through the cartridge with a gentle positive pressure from a syringe or a pressure manifold. Attempt to elute the cartridge at a flow rate of about 15 to 20 drops/min (0.5 to 0.7 ml/min). Collect the eluate into a clean 13-ml graduated centrifuge tube. A small quantity of water will probably be observed in the bottom of the tube.

Note: Do not use vacuum to elute the methyl *tert*-butyl ether through the cartridge.

12. Transfer the eluate from the centrifuge tube to a drying tube with a disposable Pasteur pipet (see [Figure 3](#)). Try not to transfer the water from the bottom of the tube. Allow the methyl *tert*-butyl ether solution to percolate through the drying tube and into a 5-ml graduated centrifuge tube.
13. Rinse the 13-ml centrifuge tube twice with 0.50-ml portions of fresh methyl *tert*-butyl ether. Transfer the rinses to the drying tube. Allow the rinses to percolate through the drying tube and into a 5-ml graduated centrifuge tube. Expel the last traces of solvent from the drying tube into the 5-ml graduated centrifuge tube with a pipet bulb. Rinse the drying tube with 0.50 ml of fresh methyl *tert*-butyl ether, and once again expel the last of the solvent from the drying tube into the 5-ml centrifuge tube.
14. Dilute the solution in the 5-ml centrifuge tube to 5.0 ml with methyl *tert*-butyl ether.

Note: This solution can be refrigerated ( $4\text{EC} \pm 3\text{EC}$ ) for 2 weeks before analysis. Dilute the solution to 5.0 ml with methyl *tert*-butyl ether before analysis. Because of the volatility of the methyl *tert*-butyl ether, add the solution to the autosampler vials just prior to gc/ms analysis.

15. Place 0.5-ml to 0.7-ml aliquots of the solution from step 14 into gc autosampler vials. Seal the vials with suitable septa. Label one vial repetition 1 and the other repetition 2.

### 3.2.2.5 Analysis by Gas Chromatography/Mass Spectroscopy (gc/ms-sim)

#### 3.2.2.5.1 Standard Procedure

##### A. Instrument Conditions:

Injector: Splitless mode, 200<sup>B</sup>C, purge off time 0.75 min.

Column: Fused silica capillary columns: 0.20 mm i.d. x 12 m, methyl silicone, 0.33  $\mu\text{m}$  film thickness.

Carrier gas: Helium, 8 psi (33.5 cm/sec flow rate).

Temperatures: Hold at 55<sup>B</sup>C for 1.7 min,  
Ramp at 15<sup>B</sup>C/min to 150<sup>B</sup>C.  
Ramp at 25<sup>B</sup>C/min to 250<sup>B</sup>C.

Detector: Mass selective detector in selected ion mode. Data are acquired for ions of *m/z* 138, 207, and 249. These data are summed to give a

total ion chromatogram (TIC). The TIC data are used for calculations. Data are processed using Hewlett Packard software.

B. Procedure:

1. Place 0.5-ml to 0.7-ml aliquots of the 0.50 ppm standard (see [3.1.3.1](#)) into gc autosampler vials. Seal the vials with suitable septa.
2. Inject 1.0  $\mu$ l from the first vial of the 0.50 ppm standard.
3. Inject 1.0  $\mu$ l of the derivatized sample (see [3.2.2.4 step 15](#)) from the vial labeled repetition 1.
4. Inject 1.0  $\mu$ l from the second vial of the 0.50 ppm standard.
5. Inject 1.0  $\mu$ l of the derivatized sample (see [3.2.2.4 step 15](#)) from the vial labeled repetition 2.
6. Inject 1.0  $\mu$ l from the third vial of the 0.50 ppm standard.
7. Inject 1.0  $\mu$ l of methyl *tert*-butyl ether as a blank.
8. Compare the peak area of the sample to those of the 0.50 ppm standards on either side. If the peak area for the sample is greater than the peak areas for the 0.50 ppm standards, then prepare new aliquots of the sample (see [3.2.2.4 step 15](#)), and repeat steps 1 through 7 using the 2.50 ppm standard (see [3.1.3.1](#)) instead of the 0.50 ppm standard. If the peak area for the sample is greater than the peak areas for the 2.50 ppm standards, repeat steps 1 through 7 with the 25.0 ppm standard (see [3.1.3.1](#)).
9. Compare the peak areas of the standards injected before and after both repetitions of the sample. If the peak areas of the standards vary by >20%, prepare two new aliquots of the sample (see [3.2.2.4 step 15](#)), and repeat steps 1 through 8. If there is <20% variation, proceed to part C.

C. Calculations:

1. Compare the gc retention times with those of the 0.50 ppm standards on either side. If the gc retention time for the sample is within  $\pm 0.05$  min of either standard, then proceed to step 2.
2. Calculate ppm of FOE 5043 equivalents in each sample by comparing the detector response (peak areas) for the sample to the average response to the standards injected before and after the sample.

$$\text{Sample ppm} = \frac{\text{sample response}}{\text{average standard response}} \times \text{standard concentration}$$

D. Detector Linearity Curves:

1. To demonstrate detector response linearity to the trifluoroacetamide derivative in solvent, sequentially inject 1.0  $\mu\text{l}$  of each of the 0.10 ppm, 0.25 ppm, 0.50 ppm, 1.00 ppm, and 2.50 ppm standards (see 3.1.3.1).
2. To demonstrate detector response linearity to the trifluoroacetamide derivative in the presence of matrix, prepare and analyze fortified matrix controls. Using a 500- $\mu\text{l}$  gas tight syringe, add 0.50 ml aliquots of control matrix sample (obtained by processing a control tissue sample through the method to 3.2.2.4 step 14) to each of five auto sampler vials. Label one each of the vials 0.10 ppm, 0.25 ppm, 0.50 ppm, 1.00 ppm, and 2.50 ppm, respectively. In the same order, fortify the five vials with 2.0  $\mu\text{l}$ , 5.0  $\mu\text{l}$ , 10.2  $\mu\text{l}$ , 21.2  $\mu\text{l}$ , and 55.5  $\mu\text{l}$ , respectively, of the 25.0 ppm standard (see 3.1.3.1). These fortifications represent final concentrations of 0.10 ppm, 0.25 ppm, 0.50 ppm, 1.00 ppm, and 2.50 ppm, respectively. Inject 1.0  $\mu\text{l}$  of each fortified matrix sample.
3. Plot the TIC mass selective detector response as a function of the standard concentration (ppm) for the data from steps 1 and 2. Assess the linearity of each curve by a least squares determination (such a determination is described by Aiken *et al.*<sup>8</sup>). To be considered linear, each curve should have a correlation coefficient (r) of  $\geq 0.99$ .
4. Assess the two curves plotted in step 3. Determine detector response values from each curve at 0.10 ppm, 0.25 ppm, 0.50 ppm, and 1.00 ppm. At each of these points, the detector response value for the fortified matrix curve should be within 20% of the same point on the solvent only sample curve for the curves to be sufficiently comparable.
5. If the linearity of the two curves is not sufficient, repeat steps 1 to 5 after evaluating the performance of the gc/ms system.

Note: If standards greater than 0.50 ppm were necessary in step 3.2.2.5.1 B-8, use correspondingly higher linearity curves (ie. 1.00 ppm, 2.50 ppm, 5.00 ppm, 10.0 ppm, and 25.0 ppm with 1.00 ppm recoveries and fortify the control matrix with 250 ppm standard). For recoveries lower than 0.10, use a correspondingly lower linearity curve.

3.2.2.5.2 Confirmatory Procedure

1. Individually integrate the single ion chromatograms for 138, 207, and 249 amu.
2. Compare the ratio of peak areas for 138 amu to 207 amu and 249 amu to 207 amu for the standards and the samples.

$$\text{138 Ion Ratio} = \frac{\text{Integration for 138 amu}}{\text{Integration for 207 amu}}$$

$$\text{249 Ion Ratio} = \frac{\text{Integration for 249 amu}}{\text{Integration for 207 amu}}$$

3. If the ion ratios for the sample are similar ( $\pm 15\%$ ) to the average ion ratios of the standards injected before and after the sample, the presence of FOE 5043 residue, measured as the trifluoroacetamide derivative, is confirmed. See [Appendix 3](#) for sample calculations.

#### 3.2.2.6 Disposal of Solutions

1. All organic solvent waste (methylene chloride, acetonitrile, methyl *tert*-butyl ether, and the aqueous elutes from C-18 cartridge cleanup) should be disposed of in approved hazardous waste containers.
2. The aqueous wastes (the aqueous phase from partitioning) should be disposed into an approved water waste system.
3. Pot residues from the steam distillation should be diluted with water (about 300 ml), neutralized to about pH 7 with 37% hydrochloric acid, and emptied into an approved water waste system. Flush drain lines with a copious quantity of water.

### 3.3 Method Validation

#### 3.3.1 Requirements

1. Duplicate recoveries of 70 to 120% in all plant matrices and processed products at 0.10 ppm for FOE 5043, FOE oxalate, FOE sulfonic acid, and FOE thioglycolate sulfoxide are required.
2. Each gc/ms measurement of a 0.10 ppm recovery is compared to a 0.50 ppm standard to determine the ppm level of FOE 5043 equivalents of residue. (0.25 ppm standard should be used for 0.05 ppm recoveries.)

3. Each sample is analyzed by the confirmatory method.
4. Calculate recoveries by the following equation:

$$\text{Recovery} = \frac{\text{Ppm found}}{\text{Ppm fortification level}} \times 100\%$$

### 3.3.2 Procedure for 0.10 ppm recoveries

1. Using a 250 µl gas tight syringe, add 0.200 ml of FOE 5043 standard solution (5.00 µg/ml in methanol, see [3.1.3.2](#)); 0.124 ml of FOE oxalate (5.00 µg/ml in methanol, see [3.1.3.2](#)); 0.173 ml of FOE sulfonic acid, sodium salt, monohydrate (5.00 µg/ml in methanol, see [3.1.3.2](#)); or 0.166 ml of FOE thioglycolate sulfoxide (5.00 µg/ml in methanol, see [3.1.3.2](#)) to the 1000-ml flask ([3.2.2.2 step 1](#)).

Note: If the FOE sulfonic acid is in the form of the free acid, use 0.151 ml of 5.00 µg/ml solution. For 0.05 ppm recoveries, use half the volumes of fortification solution.

2. Run duplicate recoveries for each chemical in each matrix. Run duplicates sample for each matrix. Run a control sample with each matrix set.
3. Perform the method as written without modifications.

## 4.0 Results and Discussion

Hydrolysis of FOE 5043 and its plant metabolites yields a common chemical fragment, fluoroaniline (see [Appendix 4](#)). Thus, a common residue method was developed. A flow diagram of the analytical residue method is presented in [Figure 4](#).

### 4.1 Sample Oxidation and Hydrolysis

A 24-hour hydrolysis with refluxing 47% sulfuric acid completely converted FOE 5043, FOE oxalate, FOE sulfonic acid, FOE methyl sulfone, and FOE thioglycolate sulfone to fluoroaniline. Lower concentrations of acid or shorter reflux times did not hydrolyze all of the plant metabolites to the fluoroaniline. Higher concentrations of acid (>60%) degraded the fluoroaniline. Alkaline hydrolysis was also attempted, but a significant fraction of the highly volatile fluoroaniline was lost during the hydrolysis.

Under the acidic conditions, FOE thioglycolate sulfoxide and FOE methyl sulfoxide did not hydrolyze completely to the fluoroaniline, so the residue samples were oxidized in order to convert the sulfoxide metabolites to the corresponding sulfones prior to the acid hydrolysis.