Report Title : Fomesafen : Determination of Fomesafen in Soil or Water

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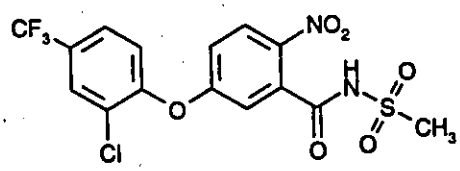
1 Scope

This method is intended for the determination of residues of fomesafen at concentrations of 10 to 1240 µg/kg (ppb) in soil and 1 to 100 µg/L (ppb) in water. The Chemical Abstracts Name and Registry Number for fomesafen is 5-[2-chloro-4-(trifluoromethyl)phenoxy]-N-methylsulfonyl)-2-nitrobenzamide (9CI) [72178-02-0]. The chemical structure is given below.



2 Summary

A known weight of soil is extracted with a mixture of methylene chloride and acidified water, or a known volume of water is acidified and extracted with methylene chloride. In each case, a measured portion of the methylene chloride extract is evaporated to dryness and dissolved in mobile phase for final determination by high performance liquid chromatography (HPLC) with detection at 290 nm.

3 Materials/Methods

The equipment and reagents described below were used to generate the data and chromatograms presented in this report. Equipment capable of providing equivalent sensitivity and selectivity, and reagents of comparable purity can be used.

3.1 Apparatus

1. Liquid Chromatograph

   Hewlett-Packard (HP) Model 1090II equipped with diode array detector, an automatic injector and a HPLC^{3D} ChemStati on DOS Series data acquisition system. Instrument with the above specifications is available from Hewlett-Packard Company, Wilmington, DE.

2. Analytical Column

   Spherisorb ODS-2, 5 µm particle size, 100A pore, 150 mm long x 4.6 mm i.d., Alltech Associates, Inc. Cat No. 8545, S/N 94021359.

3. Analytical Balance

   Sartorius Analytic Model A200S, or equivalent.

4. Solvent Filtering Unit

   Millipore All-Glass Filter Holder for 47 mm diameter filters, catalogue No. XX15 047 00, equipped with Millipore 0.45 µm pore size HV filter for vacuum filtering of HPLC solvents.

5. Ultrasonic Cleaner

   Branson Model No. B-22-4, stainless-steel tank 2.8 liter capacity.

6. Laboratory Shaker

   Eberbach 6010 two-speed reciprocating shaker with utility box carrier (VWR catalogue No. 57007-101).

7. Vortex Mixer

   Thermolyne Maxi-Mix™ vortex mixer with push-button on/off switch, VWR catalogue No. 58810-163.

8. Nylon Filter

   Disposable Nylon Acrodisc 13 HPLC syringe filter, 0.45 µm pore, 13 mm diameter, Gelman Catalogue No. 4426.

9. Disposable Syringe

   B-D Plastipak disposable syringe with Luer-Lok end, 3-mL capacity, B-D Catalogue No. 9585.

10. Disposable Centrifuge Tube

    Polypropylene, sterile, clear Corning brand, disposable conical centrifuge tube, 15 mL-capacity, Corning No. 430766.

11. Glass Bottles

    8-oz clear wide-mouth, with polytetrafluoroethylene(PTFE)-lined caps for soil samples; 16-oz clear narrow-mouth, with PTFE-lined caps for water samples; 1-oz narrow-mouth with Polyseal-lined caps.

12. Bottle-Top Dispenser

    Brinkman Dispensette bottle-top dispenser, adjustable 10-50 mL volume, VWR Catalogue No. 53519-825.

13. Pasteur disposable glass transfer pipettes.

14. Solid Phase Extraction (SPE) Columns

    J&W Accubond® Silica SPE cartridges for extract cleanup, 3 mL size, 500 mg silica, J&W Part No. 188-0150.

15. Vacuum Manifold

    J&W vacuum manifold, 12-place glass basin system with lid, vacuum gauge and bleed valve, J& W Scientific No. 600-4000.

3.2 Reagents and Standards

1. Water

   HPLC grade, Fisher Scientific Catalogue No. W5-4.

2. Methylene Chloride

   Optima grade, Fisher Scientific Catalogue No. D151-4.

3. Ethyl Acetate

   Optima grade, Fisher Scientific Catalogue No. E196-4.

4. Glacial Acetic Acid

   Certified ACS grade, Fisher Scientific Catalogue No. A38C-212.

5. Acetonitrile

   HPLC grade, Burdick & Jackson Brand from Baxter, Catalogue No.015-4.

6. Potassium Nitrate

   Analytical Reagent grade, Mallinckrodt Catalogue No. 7028.

7. Phosphoric Acid

   Reagent grade, 85% .

8. Sodium Sulfate

   Anhydrous, Certified ACS grade

9. Fomesafen Analytical Reference Standard

   Zeneca Analytical Standard ASJ10035-01S, 98.3% w/w purity or equivalent, available from Zeneca Ag Products, 1200 South 47th Street, Richmond, CA 94804-4610.

3.2.1 Preparation of Mobile Phase Solutions

1. Mobile Phase Solution A (acetonitrile:water, 10:90)

   Dissolve 1.01 g of potassium nitrate in 900 mL of HPLC grade water. Adjust to pH 3 with phosphoric acid as indicated by pH sticks. Add 100 mL of HPLC grade acetonitrile. Mix well and vacuum filter through a Millipore 0.45 µm pore size HV filter prior to use.

2. Mobile Phase Solution B (acetonitrile:water, 90:10)

   Dissolve 1.01 g of potassium nitrate in 100 mL of HPLC grade water. Adjust to pH 3 with phosphoric acid as indicated by pH sticks. Add 900 mL of HPLC grade acetonitrile. Mix well and vacuum filter through a Millipore 0.45 µm pore size HV filter prior to use.

3. Mobile Phase Solution C (acetonitrile:water, 30:70)

   Dissolve 0.700 g of potassium nitrate in 700 mL of HPLC grade water. Adjust to pH 3 with phosphoric acid as indicated by pH sticks. Add 300 mL of HPLC grade acetonitrile. Mix well and vacuum filter through a Millipore 0.45 µm pore size HV filter prior to use.

3.2.2 Preparation of Calibration Standard Solutions

To prepare a stock calibration solution at a concentration of 1.0 mg/mL, weigh accurately a known quantity (50 mg ± 2 mg) of primary standard fomesafen of known purity into a clean beaker. Add a sufficient volume of acetonitrile to the beaker to dissolve the fomesafen. Quantitatively transfer the fomesafen solution to a clean 50-mL volumetric flask, and dilute to volume. Stopper the volumetric flask and mix the contents thoroughly. Calculate the concentration of the stock solution as follows:

$$C = \frac{W \times P}{50}$$

Where

C = the concentration of fomesafen in final solution (mg/mL)

W = the weight of primary standard taken (mg)

P = the purity of the primary standard

50 = the volume of solvent (mL)

Transfer the contents into a glass bottle. Cap the bottle with a Polyseal-lined cap, and keep refrigerated when not in use.

To prepare working standard solutions for calibration purposes, dilute the stock calibration solution with Mobile Phase Solution C (acetonitrile:water 30:70) to give 10, 5, 2, 1, 0.5, 0.2 and 0.05 µg/mL solutions. Transfer working standard solutions to glass bottles with Polyseal-lined caps and keep refrigerated when not in use.

3.2.3 Preparation of Fortification Standard Solutions

To prepare a stock fortification standard solution at a concentration of 1250 µg/mL, weigh accurately a known quantity (127 mg ± 2 mg) of primary standard fomesafen of known purity into a clean beaker. Add 50 mL of acetonitrile to the beaker to dissolve the fomesafen. Quantitatively transfer the fomesafen solution to a clean 100-mL volumetric flask, and dilute to volume with water to volume. Stopper the volumetric flask, and mix the contents thoroughly. Prepare diluted fortification solutions (125 µg/mL) by diluting appropriate portions of the stock fortification solution with acetonitrile:water 1:1 solvent mixture. Transfer solutions to glass bottles with Polyseal-lined caps and keep refrigerated when not in use.

3.3 Analytical Procedure

3.3.1 Preparation of Fortified Samples

Fortified and unfortified control samples are analyzed with each sample set to demonstrate method recovery and performance. Fortify 50-g portions of soil samples or 400-mL aliquot of water samples by adding known volumes of the fortification standard solution of fomesafen (as prepared in Section 3.2.3) to the control samples before extraction. Extract the fortified samples as detailed below.

3.3.2 Extraction of Samples

For soil samples, transfer a 50-g portion of well mixed soil into an 8-oz wide-mouth bottle. Add 50 ± 1 mL of water, and add minimum 0.5 ± 0.1 mL of glacial acetic acid. Use a bottle-top dispenser to add 50 ± 0.1 mL methylene chloride. Cap the bottle securely with a PTFE-lined cap and shake to mix. Use a pH stick to measure the resulting pH of the mixture. Add additional glacial acetic acid, if necessary, to ensure that the resulting pH is ~4.5 or lower. Shake the bottle and its content on a reciprocating shaker for 60 minutes. Centrifuge for 20 minutes to separate the phases. Remove 12-15 mL of the middle layer of methylene chloride extract using a disposable pipette and transfer into a 1-oz bottle. Add sodium sulfate (~1 g or more) to dry the extract. Close the bottle with a Polyseal-lined cap and swirl to mix the contents.

For water samples, transfer a 400-mL aliquot of water into a 16-oz narrow-mouth bottle. Add 1.0 ± 0.1 mL of glacial acetic acid. Use a bottle-top dispenser to add 25 ± 0.1 mL methylene chloride. Cap the bottle securely with a PTFE-lined cap and shake to mix. Use a pH stick to measure the resulting pH of the aqueous portion of the mixture. Add additional glacial acetic acid, if necessary, to ensure that the resulting pH is ~4.5 or lower. Shake the bottle and its content on a reciprocating shaker for 60 minutes. Place the bottle in its upright position for phase separation or if necessary, centrifuge. Use a disposable pipette to transfer 12-15 mL of the bottom layer of methylene chloride extract into a 1-oz bottle. Add sodium sulfate (~1 g or more) to remove water. Close the bottle with a Polyseal-lined cap and swirl to mix the contents.

3.3.3 Concentration of Extracts

Pipette a 10.0 mL aliquot of the dried methylene chloride extract from Section 3.3.2 into a 15-mL polypropylene centrifuge tube. Evaporate to dryness under a stream of dry air at room temperature (~25°C) and dissolve the residuum in 1.0 mL of Mobile Phase Solution C. Cap the tube and vortex mix for 1 minute, followed by 15 minutes of sonication in an ultrasonic cleaner. Repeat this vortex mix / sonication step for a total of 2 times. Filter the final extract through a 0.45 µm filter attached to a 3-mL disposable syringe into an autosampler for HPLC analysis. Final sample-to-solvent ratio is 10 g/mL for soil and 160 mL/mL for water.

3.3.4 Cleanup of Extracts

The methylene chloride extracts from soil or water samples generally do not require column cleanup. However, if peak detection and identification are prevented due to interferences, the methylene chloride extract may need to undergo silica cleanup as described in the following procedure.

Connect an SPE cartridge (J&W Accubond) packed with silica to a vacuum manifold. Pre-wash the Silica cartridge with 2.5 mL of methylene chloride and discard the methylene chloride. Transfer a 10.0 mL aliquot of the dried methylene extract from Section 3.3.2 and allow the extract to pass through the cartridge. Use the vacuum manifold to aid the process. Discard the eluate. Wash the cartridge with 2 mL of methylene chloride and discard the washing. Elute the cartridge with 9 mL of ethyl acetate; collect the eluate in a 15-mL centrifuge tube. Evaporate the ethyl acetate to dryness under a stream of dry air and

dissolve the residuum in 1.0 mL of Mobile Phase Solution C. Cap the tube and vortex mix for 1 minute, followed by 15 minutes of sonication in a ultrasonic cleaner. Repeat this vortex mix/sonication step for a total of 2 times. Filter the final extract through a 0.45 µm filter attached to a 3-mL disposable syringe into an autosampler for HPLC analysis.

3.3.5 High Performance Liquid Chromatographic Conditions

Instrument:	Hewlett-Packard (HP) Model 1090II equipped with diode array detector and an automatic injector
Column:	Spherisorb ODS-2, 5 µm, 100A pore, 150 mm long x 4.6 mm i.d., from Alltech Associates, Inc., Cat. No. 8545, S/N 94021359
Mobile Phases:	A Acetonitrile:Water, 10:90, 0.01N KNO_3 to pH 3 B Acetonitrile:Water, 90:10, 0.01N KNO_3 to pH 3
Data Acquisition:	HP HPLC^{3D} ChemStation DOS Series
Flow Rate:	0.75 mL/min 65% Mobile Phase A 35% Mobile Phase B

Time Table:

Time (min)	%B
0.00	35
7.00	100
7.50	100
8.00	35
11.00	35

Column Temperature:	60 °C
Detector Wavelength:	290 nm
Injection Volume:	75 µL
Sampling Interval:	0.640 sec
Run Time:	11 min

Using the above conditions, the elution time for fomesafen was 6.6 minutes. See Figures 1 to 5 for typical chromatograms.

3.3.6 Calibration

Calibrate the liquid chromatograph with the daily-use calibration standards. Inject the entire range of solutions, from 0.05 µg/mL to 10 µg/mL, at the beginning and at the end of each run. After every 6 to 8 samples, inject one or more of the calibration standards to assure that the fomesafen response is stable.

3.3.7 Analysis of Sample Extracts

Analyze the final mobile phase extract from each soil/water sample on the same day of calibration. Inject the sample extracts using the same conditions and injection volumes as those used for the calibration standards. The identity of the fomesafen peak in the sample chromatogram is assigned based upon the coincidence of the retention time (± 0.10 minute) with that of the fomesafen peak in the calibration standard chromatogram. Dilute the extract with Mobile Phase Solution C, if necessary, to keep the fomesafen response within the calibration range.

3.4 Calculations

The concentration of fomesafen in the original sample is calculated by using the external standard method; that is, the response obtained for fomesafen in the sample extract is compared to the response obtained from a separate injection of fomesafen calibration solution. To use the linear response calculation method shown below, the injection volumes for all calibration solutions and sample extracts must be fixed at the same volume.

3.4.1 Calibration Response Factor

Calculate the response factor, RF, for injection of a calibration solution as follows:

$$RF = \frac{C_{std}}{R_{std}}$$

Where

C_{std} = concentration in µg/mL of the calibration solution

R_{std} = response units (for example, peak height, peak area, or electronic units) from detector for the calibration solution

3.4.2 Fomesafen in Sample

Determine the concentration of fomesafen in the original sample, C_s (in µg/kg or µg/L), from the average response factor, RF_{avg}, and the sample response, R_{sample}, as follows:

$$C_s \text{ (µg/kg or µg/L)} = \frac{R_{sample} \times RF_{avg} \times D \times 10^3}{C}$$

Where

R_{sample} = response unit from detector for the sample final extract

RF_{avg} = average response factor over the entire range of calibration

C = concentration of sample in final extract (sample-to-solvent ratio, in g/mL or mL/mL) = 10 g/mL for soil or 160 mL/mL for water

D = dilution factor required if final extract is diluted to keep in calibration range