Report Title: Fomesafen: Determination of Fomesafen in Water by Liquid-Solid Extraction and High Performance Liquid Chromatography with Ultraviolet Detection

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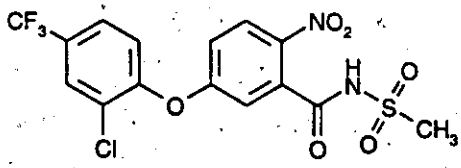
1 Scope

This method is intended for the determination of residues of fomesafen in drinking water and surface water sources. The limit of quantitation for this method is 0.1 µg/L (ppb) in water. The method has been validated for trace analysis of fomesafen at concentrations of 0.1 to 10 µg/L in water. The Chemical Abstracts Name for fomesafen is 5-[2-chloro-4-(trifluoromethyl)phenoxy]-N-methylsulfonyl)-2-nitrobenzamide (9Cl), CAS No. 72178-02-0. The chemical structure is given below.



2 Summary

A measured volume of water of 500 mL is extracted using a C18 liquid-solid extraction disk. Fomesafen residue is eluted from the disk using methanol. The methanol eluate is evaporated to dryness and re-dissolved in acetonitrile/water mobile phase for final determination by high performance liquid chromatography (HPLC) with UV 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

3.1.1 Liquid Chromatograph

Hewlett-Packard (HP) Model 1090 equipped with a UV diode array detector (DAD), an automatic injector, 250-µL injection volume option, and a HPLC 3D ChemStation DOS Series data acquisition system. Instrument with the above specifications is available from Hewlett-Packard Company, Wilmington, DE.

3.1.2 Injection Volume Modification for HP1090 HPLC (Optional)

To obtain sufficient instrumental sensitivity with detectable HPLC responses from the lowest calibration standard solution, it may be necessary to increase injection volume in excess of 250 µL. The following modifications are required to allow injections up to 500 µL. Other instruments may require different (or no) modifications.

Replace 250-µL syringe with a 500-µL Hamilton series 1700 gas-tight syringe (Hamilton #81230). The waste sleeve hole for the 250-µL syringe plunger will have to be enlarged to accommodate the larger diameter of the 500-µL syringe plunger (HP 79846-24502). Increase the total volume of the injection loop to at least 500 µL by adding an additional 250-µL sample loop (HP #79846-87613) between the existing loop and the auto-injector needle. Note: It is important to remember that the HP ChemStation software controlling the HPLC does not recognize injection volumes greater than 250 µL. After this modification is made all injection volumes are actually two times (2X) the volume entered in the ChemStation method.

3.1.3 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.

3.1.4 Analytical Balance

Sartorius Analytic Model A200S, capable of accurately weighing 0.0001g.

3.1.5 Extraction Apparatus

Liquid-solid extraction disks – C18 (Octadecyl) Empore™ extraction disk, 47 mm, 3M catalog No. 98050300155.
Vacuum pump – Welch® GEM™ Model 8890A vacuum pump.
Millipore standard filter apparatus – All glass, to hold extraction disk.

3.1.6 Vacuum Rotary Evaporator

Buchi Model assemblies "RE-121 Rotovapor" with thermostatically controlled water bath, for evaporating methanol eluate in 50-mL round-bottom flask.

3.1.7 Solvent Filtering Unit

Millipore All-Glass Filter Holder for 47 mm diameter filters, catalog No. XX15 047 00, equipped with Millipore 0.45 µm pore size HV filter, catalog No. HVLP 04700, for vacuum filtering of HPLC solvents.

3.1.8 Glass Flasks

50-mL capacity, round-bottom, Kimax No. 25285 for collecting the methanol eluate.

3.1.9 Glass Bottles

16-oz clear narrow-mouth, with PTFE-lined caps for collecting water samples; 1-, 2-, and 4-oz capacity, narrow-mouth, with PolySeal®-lined screw caps, for storing standard solutions.

3.1.10 Pasteur disposable glass transfer pipettes

3.1.11 Glass Pipettes

1-, 2-, 5-, and 10-mL disposable, graduated glass pipettes for general use (Kimble).

3.1.12 Syringes

10-, 25-, 100-, and 250-µL capacity (Hamilton gas-tight 1700 series) for sample fortifications.

3.1.13 pH Indicator Strips

EM Science ColorpHast® strips, 0 to 6 pH range, catalog No. EM-9586-3.

3.2 Reagents and Standards

3.2.1 Water

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

3.2.2 Methanol

HPLC grade or High Purity Solvent (supplied by Burdick & Jackson Catalog No. 230-4).

3.2.3 Glacial Acetic Acid

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

3.2.4 Acetonitrile

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

3.2.5 Potassium Nitrate

Analytical Reagent grade, Mallinckrodt Catalog No. 7028.

3.2.6 Phosphoric Acid

Reagent grade, 85% .

3.2.7 Sodium Sulfate

Anhydrous, Certified ACS grade.

3.2.8 Preparation of Mobile Phase Solutions

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.

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.

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.

3.2.9 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.

3.2.10 Calibration Standard Solutions

To prepare a stock calibration solution at a concentration of 1000 µg/mL, weigh accurately a known quantity (100 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 100-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:

$C = W \times P / 100$

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 (e.g. 1.00 for 100.0% w/w purity)
100	=	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, 0.2, 0.1 and 0.05 µg/mL solutions. Transfer working standard solutions to glass bottles with PolySeal®-lined caps and keep refrigerated when not in use.

3.2.11 Fortification Standard Solutions

Fortification standard solutions are to be used for fortification purposes and should be prepared from stock standard solutions different from those used for preparation of calibration solutions. To prepare a stock fortification standard solution at a concentration of 1000 µg/mL, weigh accurately a known quantity (100 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 100-mL volumetric flask, and dilute to volume. Stopper the volumetric flask, and mix the contents thoroughly. Prepare diluted fortification solutions (100, 10 and 1 µg/mL) by diluting appropriate portions of the stock fortification solution with mobile phase solution 'C' (Acetonitrile:water 30:70). 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 500-mL aliquot of water samples by adding known volumes of the fortification standard solution of fomesafen (as prepared in Section 3.2.11) to the control samples before extraction. For example, add 50.0 µL of the 1.0 µg/mL fortification standard solution to the water sample to produce 0.1 µg/L (ppb) fortification. Extract the fortified samples as detailed below.

3.3.2 Liquid-Solid Extraction of Water Samples

Before sample extraction, precondition the C18 Empore™ extraction disk by passing through 10 mL of methanol under vacuum at approximately 10 mL/minute. Remove the vacuum immediately after all the methanol has passed through the filter in order to prevent the filter from drying out. Pass 500 mL of water sample through the filter disk under vacuum at a rate of approximately 100 mL/min. Allow the disk to dry and discard the water.

Elute the C18 Empore™ extraction disk under vacuum with 10 mL of methanol. Collect the methanol eluate in a 50-mL round-bottom flask. Transfer the flask to a rotary evaporator and evaporate the sample to dryness with the water bath temperature set at 30°C (temperature not to exceed 40°C). Re-dissolve the residuum in 1.0 mL of Mobile Phase 'C' (Acetonitrile: water 30:70). Final sample to solvent ratio is 500 mL/mL for

water to Mobile Phase 'C'. Use a disposable glass transfer pipette to transfer the sample final extract into an autosampler vial for analysis by HPLC.

3.3.3 HPLC Conditions

	HPLC
Instrument:	Hewlett-Packard (HP) Model 1090 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
Mobile Phase :	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:	100 - 300 µL
Sampling Interval:	0.640 sec
Run Time:	11 min

Using the above conditions, the elution time for fomesafen was 7.2 to 7.3 minutes. See Figures 1 to 5 for typical chromatograms.

3.3.4 Calibration

Before starting to calibrate the chromatographic system, first made one or two injections of the 10 µg/mL standard solution to condition the inlet and column. Calibrate the liquid chromatograph with the daily-use calibration standard solutions (Section 3.2.10). Inject the entire range of solutions, from 0.05 µg/mL to 5 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.

3.3.5 Analysis of Sample Extracts

Analyze the final mobile phase extract from each 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 '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, i.e., 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:

$$RF = \frac{Cstd}{Rstd}$$

Where

Cstd = concentration in µg/mL of the calibration solution

Rstd = response units (e.g., peak height, peak area, electronic units) from detector for the calibration solution

3.4.2 Fomesafen in Sample

Determine the concentration of fomesafen in the original sample, Cs (in µg/L), from the average response factor, RFavg, and the sample response, Rsample, as follows:

$$Cs\ (\mu g/L) = \frac{Rsample \times RFavg \times D \times 10^3}{C}$$

Where

Rsample = response unit from detector for the sample final extract

RFavg = average response factor over the entire range of calibration

C = Concentration of sample in final extract (sample to solvent ratio, in mL/mL) = 500 mL/mL for water

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