DowElanco

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DETERMINATION OF RESIDUES OF DE-498 IN SOIL BY CAPILLARY GAS CHROMATOGRAPHY/MASS SPECTROMETRY

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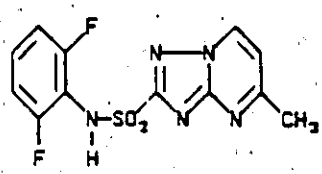
1. Scope

This method is applicable for the quantitative determination of DE-498 (N-(2,6-difluorophenyl)-5-methyl-1,2,4-triazolo-[1,5a]-pyrimidine-2-sulfonamide) in soil at a validated lower level of quantitation of 2.5 ppb.



N-(2,6-difluorophenyl)-5-methyl-1,2,4-triazolo-[1,5a]-pyrimidine-2-sulfonamide (DE-498)

2. Principle

DE-498 residues are extracted from soil using a 90% acetone/10% 0.1 N hydrochloric acid solution. Following evaporation of the acetone, the sample is diluted with 0.005 N hydrochloric acid and purified using a C_{18} solid-phase extraction (S-P-E). The eluent from the S-P-E is evaporated to dryness, and the residue reconstituted with acetonitrile. The sample is then derivatized with methyl iodide to form the N-methyl derivative. The derivatized sample solution is evaporated to dryness, reconstituted with toluene containing N-d3-methyl DE-498 as an internal standard and analyzed by capillary gas chromatography/mass spectrometry GC/MS

3. Safety Precautions

a. Each analyst should be acquainted with the potential hazards of the reagents, products, and solvents used in this method before commencing laboratory work. SOURCES OF INFORMATION INCLUDE: MATERIAL SAFETY DATA SHEETS, LITERATURE, AND OTHER RELATED DATA. Safety information on non-DowElanco products should be requested from the supplier. Disposal of reagents, reactants, and solvents must be in compliance with local, state, and federal laws and regulations.

b. Acetone, acetonitrile, methanol, methyl-t-butyl ether, toluene, and triethylamine are flammable and should be used in well-ventilated areas away from ignition sources.

4. Equipment

a. Gas chromatograph, Model 5890A, Hewlett-Packard, Avondale, PA 19311.

b. Automatic sampler, Model 7673A, Hewlett-Packard, Avondale, PA 19311.

c. Mass selective detector, Model 5971A, Hewlett-Packard, Palo Alto, CA 94304.

d. Mass spectrometer data system, Model 59970, Hewlett-Packard, Palo Alto, CA 94304.

e. Balance, analytical, Model AE200, Mettler Instrument Corp., Hightstown, NJ 08520.

f. Balance, pan, Model BB2440, Mettler Instrument Corp.

g. Centrifuge, with head to accomodate 10-dram vials, Model Centra-8, International Equipment Company, Needham Heights, MA 02194.

h. Desiccator, 250 mm I.D. with Drierite adsorbent, Catalog Number 08-595E, Fisher Scientific, Pittsburg, PA 15219.

i. Evaporator, N-Evap, Model 111, Organomation Associates, Inc., South Berlin, MA 01549.

Set at a water bath temperature of 40°C and a nitrogen flow rate of 200 mL/min.

j. Oven, Model OV-490A-2, Blue M Electric Company, Blue Island, IL 60406.

k. Shaker, variable-speed reciprocating with box carrier, Model 6000, Eberbach Corp., Ann Arbor, MI 48106.

l. Ultrasonic bath, Model 1200, Branson Cleaning Equipment Company, Shelton, CT 06484.

m. Vacuum manifold box, Model spe-21, J. T. Baker Chemical Company, Phillipsburg, NJ 08865.

n. Vial crimper, Part Number 8710-0979, Hewlett-Packard, Avondale, PA 19311.

o. Vortex mixer, Model G-560, Scientific Industries, Inc., Bohemia, NY 11716.

5. Glassware and Materials

a. Column, capillary gas chromatography, Durabond-17 liquid phase, 10 m x 0.18 mm i.d., 0.3 µm film thickness, Catalog Number 121-1713, J&W Scientific, Folsom, CA 95630.

b. Column inlet liner, deactivated, Catalog Number 5181-3315, Hewlett-Packard, Avondale, PA 19311.

c. Column, C_{18} S-P-E, Catalog Number 7020-07, J. T. Baker Chemical Company. (Note 16.b)

d. Cylinder, graduated, 2000 mL, Catalog Number 131-9058, National Scientific Company, Lawrenceville, Georgia 30245.

e. Dish, weighing, Catalog Number 08-732, Fisher Scientific.

f. Gas, helium, 99.995% purity, Scott Specialty Gases, Troy, MI 48083.

g. Gas, nitrogen, technical grade, Scott Specialty Gases.

h. Moisture trap, Catalog Number 7971, Chrompack, Inc., Raritan, NJ 08869. (Note 16.c.)

i. Charcoal scrubber, Catalog Number 7972, Chrompack, Inc. (Note 16.c.)

j. Oxygen trap, Catalog Number 7970, Chrompack, Inc. (Note 16.c.)

k. Syringes, 10, 50, and 500 µL, Model 700 Series, Hamilton Company, Reno, NV 89520.

l. Vials, 2 dram, with poly(tetrafluoroethylene)-lined screw caps, Catalog Number B7800-3, National Scientific Company.

m. Vials, 10 dram, with poly(tetrafluoroethylene)-lined screw caps, Catalog Number B7800-6, National Scientific Company.

n. Vials, autosampler, 2 mL, Catalog Number C4011-2, National Scientific Company.

o. Vial seals, Catalog Number C4011-1A, National Scientific Company.

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6. Reagents and Chemicals

a. Acetone, acetonitrile, methanol, methyl-t-butyl ether, and toluene (Optima Grade), Fisher Scientific.

b. Hydrochloric acid, 0.1 N, reagent grade, certified concentration, Fisher Scientific.

c. Sodium chloride, ACS reagent grade, Fisher Scientific.

d. Hydrochloric acid, 0.005 N.

Prepare by diluting 50 mL of 0.1 N hydrochloric acid to volume in a 1000-mL volumetric flask with distilled/deionized water.

e. Sodium chloride, 5% (w/v).

Prepare by dissolving 50 grams of sodium chloride in distilled/deionized water in a 1000-mL volumetric flask. Adjust to volume with distilled/deionized water.

f. 90% acetone/10% 0.1 N hydrochloric acid solution.

Prepare by pouring 200 mL of 0.1 N hydrochloric acid into a 2000-mL graduated cylinder. Add 1500 mL of acetone, swirl the cylinder, and allow to equilibrate to room temperature. Adjust to volume with acetone.

g. Methyl iodide, minimum 99.5% purity, Catalog Number 28,956-6, Aldrich Chemical Company, Milwaukee, WI 53233.

h. Methyl iodide, stable-isotope labeled, $^{12}CD_3I$, Catalog Number 29,675-9, Aldrich Chemical Company.

i. Triethylamine, minimum 99% purity, Catalog Number 13,206-3, Aldrich Chemical Company.

j. Water, distilled/deionized, Corning MEGA-PURE Still, Model MP-12A, Corning Glass Works, Science Products Division, Corning, NY 14831.

k. Standard

N-(2,6-difluorophenyl)-5-methyl-1,2,4-triazolo-[1,5a]-pyrimidine-2-sulfonamide (DE-498), analytical standard.[a/]

[a/] Obtain from Sample Coordinator, DowElanco, P.O. Box 1706, Midland, Michigan 48641-1706.

7. Preparation of Standards

a. Preparation of Calibration Standards/Spiking Solutions

(1) Dissolve 0.1000 gram of DE-498 analytical standard in acetone in a 100-mL volumetric flask. Dilute to volume to obtain a 1000 µg/mL stock solution.

(2) Dilute 5 mL of the above 1000 µg/mL solution to 1000 mL with acetone in a 1000-mL volumetric flask to obtain a 5.0 µg/mL (5.0 ng/µL) initial solution.

(3) Solutions for spiking soil samples are prepared by diluting the initial solution from Section 7.a.(2) above with acetone as follows:

Aliquot of Initial Soln.	Final Soln. Volume	Spiking Soln. Final Conc.	Equivalent Sample Conc.
mL	mL	ng/mL	ppb
5.0	2000	12.5	2.5
10.0	2000	25.0	5.0
20.0	2000	50.0	10.0
50.0	2000	125.0	25.0
10.0	200	250.0	50.0
20.0	200	500.0	100.0
50.0	200	1250.0	250.0

(4) Solutions for calibration standards are prepared by pipeting 1.0 mL of the DE-498 standards in Section 7.a.(3) above into 2-dram vials and derivatizing according to the procedure described in Section 9, steps k through w.

b. Preparation of Internal Standard Solution

(1) Pipet 2.0 mL of the 1000 µg/mL DE-498 stock solution from Section 7.a.(1) into a 2-dram vial.

(2) Evaporate the solution to dryness using an N-Evap evaporator.

(3) Add 1.0 mL of acetonitrile, cap the vial, and sonicate for 5-10 seconds.

(4) Add 50 µL of triethylamine and 50 µL of stable-isotope labeled methyl iodide (Section 6.h), cap the vial, and sonicate for 5-10 seconds.

(5) Allow the mixture to react with the methyl iodide for 30 minutes at room temperature.

(6) Evaporate the solution to dryness using an N-Evap evaporator.

(7) Add 1.0 mL of a 5% sodium chloride solution, cap the vial, and sonicate for 5-10 seconds.

(8) Add 5.0 mL of methyl-t-butyl ether, cap the vial, and vortex the sample for 5-10 seconds.

(9) Centrifuge the vial for 5 minutes at 2500 rpm.

(10) Carefully transfer the methyl-t-butyl ether layer to a clean 10-dram vial.

(11) Repeat Steps 8-9 three additional times, combining the methyl-t-butyl ether layers in the 10-dram vial.

(12) Evaporate the solution to dryness using an N-Evap evaporator.

(13) Add 20 mL of acetone, cap the vial, and sonicate for 5-10 seconds.

(14) Transfer the acetone to a 200-mL volumetric flask.

(15) Rinse the 10-dram vial again with 20 mL of acetone, and transfer the acetone to the 200-mL volumetric flask.

(16) Dilute the solution to volume with acetone. This solution contains 10.0 µg/mL N-d3-methyl DE-498.

(17) Dilute 10.0 mL of the above 10.0 µg/mL solution to 1000 mL with toluene in a 1000-mL volumetric flask to obtain a 0.100 µg/mL (0.100 ng/µL) solution.

8. Gas Chromatography/Mass Spectrometry

a. Column

Install the splitless liner (5.b) and the capillary column (5.a) on the split/splitless injection port of the GC/MS following the manufacturer's recommended procedure.

b. Typical operating conditions for the determination of DE-498 by capillary GC/MS:

Instrumentation: Hewlett-Packard Model 5890A Gas Chromatograph / Model 5971A Mass Selective Detector

Column: J&W Scientific fused silica capillary Durabond-17 liquid phase
10 m x 0.18 mm I.d.
0.30 µm film thickness

Temperatures:

Column	120°C for 1.1 minutes 120°C to 325°C at 20°C/minute 325°C for 5.65 minutes
Injector	320°C
Interface	310°C

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Carrier Gas:	helium
Head Pressure	100 kPa
Linear Velocity	25 cm/sec
Injection Mode:	splitless
Purge Delay	1.0 minutes
Splitter Flow	50 mL/min
Septum Purge:	1.0 mL/min
Injection Volume:	2 µL
Ions Monitored:	N-methyl DE-498
	m/z 134 (base peak ion) (M^+-205; see Figure 1)
	m/z 142 (M^+-197; see Figure 1)
	N-d3-methyl DE-498 (internal standard)
	m/z 145 (M^+-197; see Figure 2)
Electron Multiplier:	1800 volts

c. A typical calibration curve is shown in Figure 3.

d. Typical chromatograms of a standard, control sample, and a 2.5 ppb recovery sample are shown in Figures 4-6, respectively.

9. Recovery of DE-498 from Soil

a. Weigh 5.0-gram portions of control soil into a series of 10-dram vials.

b. For preparing fortified samples, use part of the samples as controls and fortify the remaining samples by adding 1.0-mL aliquots of the appropriate spiking solutions (Section 7.a.(3)) in acetone to obtain concentrations ranging from 2.5 to 250 ppb.

c. Add 25 mL of a 90% acetone/10% 0.1 N hydrochloric acid extracting solution.

d. Cap the vial and sonicate the sample for 30-45 seconds.

e. Shake the sample for a minimum of 2 hours on a reciprocating shaker at approximately 180 excursions/minute.

f. Centrifuge the sample container for 10 minutes at 2500 rpm.

g. Transfer the acetone/hydrochloric acid solution to a clean 10-dram vial.

h. Evaporate the acetone using an N-Evap evaporator.

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i. Add 15.0 mL of 0.005 N hydrochloric acid, cap the vial, and sonicate the sample for 10-15 seconds.

j. The sample is then purified using the following S-P-E procedure (Note 16.b):

(1) Place a C_{18} S-P-E column on the vacuum manifold box.

(2) Rinse the S-P-E column with 5 mL of methanol.

(3) Condition the S-P-E column with 5 mL of 0.005 N hydrochloric acid. (Do not allow the column bed to dry.)

(4) Transfer the sample solution from Step 9.1 to the S-P-E column and, with the aid of vacuum, slowly pull the sample through the column. Without allowing the column bed to dry, wash the sample vial with a 10 mL aliquot of 0.005 N hydrochloric acid and transfer the wash to the S-P-E column.

(5) Thoroughly dry the S-P-E column by drawing air through it for approximately 45 minutes.

(6) Remove the S-P-E column from the vacuum manifold box and elute the DE-498 by passing 2.5 mL of methanol through the S-P-E column, collecting the eluent in a 2-dram vial. (Note 16.d)

k. Evaporate the solution to dryness using an N-Evap evaporator.

l. Add 500 µL of acetonitrile, cap the vial, and sonicate for 5-10 seconds.

m. Add 10 µL of triethylamine and 10 µL of methyl iodide, cap the vial, and sonicate for 5-10 seconds.

n. Allow the sample to react with the methyl iodide for 30 minutes at room temperature.

o. Evaporate the solution to dryness using an N-Evap evaporator.

p. Add 1.0 mL of a 5% sodium chloride solution, cap the vial, and sonicate for 5-10 seconds.

q. Add 5.0 mL of methyl-t-butyl ether, cap the vial, and vortex the sample for 5-10 seconds.

r. Centrifuge the vial for 5 minutes at 2500 rpm.

s. Carefully transfer the methyl-t-butyl ether layer to a 2-dram vial.

t. Evaporate the solution to dryness using an N-Evap evaporator.

u. Add 1.0 mL of toluene containing the N-d3-methyl DE-498 internal standard, cap the vial, and sonicate for 5-10 seconds.

v. Centrifuge the vial for 5 minutes at 2600 rpm.

w. Transfer the solution to a 2-mL autosampler vial. Seal the vial with a cap and crimper.

x. Analyze the sample by capillary gas chromatography/mass spectrometry.

10. Determination of Percent Recovery of DE-498

a. Inject the calibration standards described in Section 7.a.(4) and determine the peak areas at *m/z* 134 and *m/z* 142 for methylated DE-498 and at *m/z* 145 for d3-methylated DE-498.

For each standard calculate the DE-498 confirmation ratio. The average confirmation ratio for all of the calibration standards will be used to confirm the presence of DE-498 in the soil samples.

For example, using the data from Figure 4:

$$\text{Confirmation Ratio} = \frac{\text{peak area at } m/z\ 142}{\text{peak area at } m/z\ 134}$$

$$\text{Confirmation Ratio} = \frac{42425}{76598}$$

$$\text{Confirmation Ratio} = 0.55387$$

Positive confirmation of the presence of DE-498 is indicated when the confirmation ratio for the samples is in the range of ± 10% of the average found for the standards.

b. Prepare a standard curve by plotting the equivalent DE-498 concentration on the abscissa (x-axis) and the *m/z* 134/145 peak peak area ratio on the ordinate (y-axis) as shown in Figure 3. Using regression analysis, determine the equation for the curve with respect to the abscissa.

For example, using power regression with the data from Figure 3

$$Y = \text{constant} \times X^{(\text{exponent})}$$

$$X = \left(\frac{Y}{\text{constant}} \right)^{1/\text{exponent}}$$

$$\text{DE-498 Conc. (ppb)} = \left(\frac{m/z\ 134/145 \text{ peak area ratio}}{\text{constant}} \right)^{1/\text{exponent}}$$

$$\text{DE-498 Conc. (ppb)} = \left(\frac{m/z\ 134/145 \text{ peak area ratio}}{0.089690} \right)^{1/0.963885}$$

c. Determine the net concentration in each recovery sample by first subtracting the average DE-498 peak area ratio in the control samples from that of the recovery sample. Substitute the peak area ratio obtained into the above equation and solve for the concentration.

$$\text{DE-498 Conc. (ppb)} = \left(\frac{\text{net m/z 134/145 peak area ratio}}{0.089690} \right)^{1/0.963885}$$

$$\text{DE-498 Conc. (ppb)} = \left(\frac{0.210707-0.000000}{0.089690} \right)^{1/0.963885}$$

DE-498 Conc. = 2.43 ppb

d. Determine the percent recovery by dividing the net concentration of each recovery sample by the theoretical concentration added.

$$\text{Recovery} = \frac{\text{Concentration Found}}{\text{Concentration Added}} \times 100\%$$

$$\text{Recovery} = \frac{2.43 \text{ ppb}}{2.50 \text{ ppb}} \times 100\%$$

Recovery = 97%

11. Determination of DE-498 in Soil Samples

a. Prepare control, recovery, and treated samples as described in Section 9.

b. Prepare a standard curve and determine the DE-498 concentration in the recovery samples as described in Section 10.

c. Determine the concentration in each treated sample by substituting the DE-498 m/z 134/145 peak area ratio obtained into the equation for the standard curve and solve for the concentration.

For example, using the data from Figure 6:

$$\text{DE-498 Conc. (ppb)} = \left(\frac{\text{m/z 134/145 peak area ratio}}{\text{constant}} \right)^{1/\text{exponent}}$$

$$\text{DE-498 Conc. (ppb)} = \left(\frac{0.210707}{0.089690} \right)^{1/0.963885}$$

DE-498 Conc. = 2.43 ppb