DETERMINATION OF 2,4-DICHLOROPHENOXYACETIC ACID 2-ETHYLHEXYL ESTER, 2,4-DICHLOROPHENOXYACETIC ACID DIMETHYLAMINE SALT AS ITS 2,4-D ACID EQUIVALENT, 2,4-DICHLOROPHENOXYACETIC ACID, 2,4-DICHLOROPHENOL, 2,4-DICHLOROANISOLE, 4-CHLOROPHENOL, AND 4-CHLORPHENOXYACETIC ACID IN WATER BY GAS CHROMATOGRAPHY/MASS SELECTIVE DETECTION

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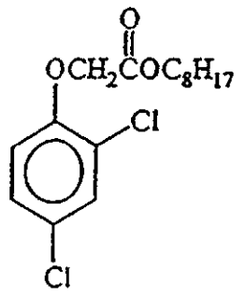
AGVISE Study No. RES944226 122

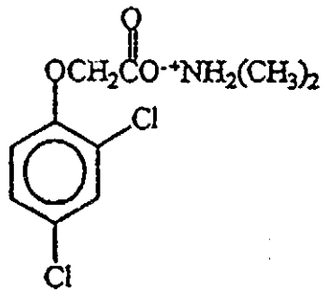
1. Scope

This method is applicable for the quantitative determination of 2,4-dichlorophenoxyacetic acid 2-ethylhexyl ester(2,4-D 2-EHE), 2,4-dichlorophenoxyacetic acid dimethylamine salt(2,4-D DMAS) as its acid equivalent, 2,4-dichlorophenoxyacetic acid(2,4-D), 2,4-dichlorophenol(2,4-DCP), 2,4-dichloroanisole(2,4-DCA), 4-chlorophenol (4-CP), and 4-chlorophenoxyacetic acid(4-CPA) in water with a Limit of Quantitation(LOQ) of 0.001 ppm.

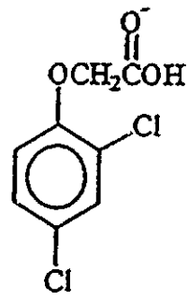
2. Structures



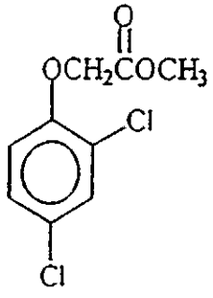
2,4-D 2-EHE



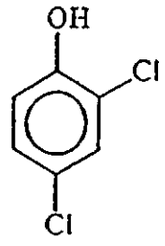
2,4-D DMAS



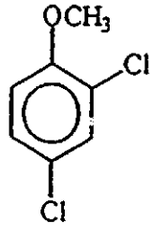
2,4-D



2,4-D ME

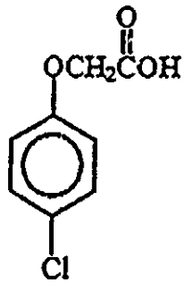


2,4-DCP

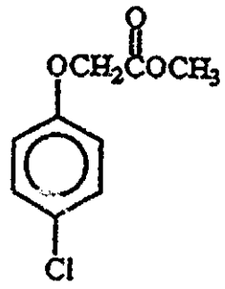


2,4-DCA

4-CP



4-CPA



4-CPA ME

3. Principle

Residues of the above analytes are extracted from water after the water solutions are acidified. The analytes are concentrated on a C18 solid phase extraction (SPE) cartridge. The analytes are eluted sequentially from the cartridge using two specific solvent systems which separate the analytes into two fractions. The first fraction contains 2,4-D 2-EHE, 2,4-DCP, and 2,4-DCA and 4-CP, which are chromatographed without derivatization. The second fraction contains 2,4-D and 4-CPA which are methylated using BF_3/methanol to form their methyl esters 2,4-D ME and 4-CPA ME, then, partitioned into toluene prior to chromatographic analysis. The first fraction and the toluene solution are combined into a single solution for injection. Quantitation is by Gas Chromatography/Mass Selective Detection in the selected ion monitoring mode (SIM). The residues are quantitated by comparing the peak areas of the test samples with the peak areas of a series of calibration standards prepared from known analytical standards.

4. Safety Precautions

4.1 Each analyst should be familiar 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 (MSDS), PRODUCT INFORMATION, and other related materials. Safety information on products should be requested from the supplier. Disposal of the reagents, reactants, and solvents must be in compliance with local, state, and federal laws and regulations.

4.2 Acetone, acetonitrile, hexane, methanol and toluene are flammable and should be used in well ventilated areas away from ignition sources.

5. Equipment(Note 15.1)

5.1 Analytical Evaporator, Meyer N-Evap, model 111, Organomation, P.O. Box 159, South Berlin, MA 01549.

5.2 Automatic Sampler, model 7673, Hewlett-Packard, P.O. Box 900, Route 41 and Starr Road, Avondale, PA 19311-9990.

5.3 Centrifuge, IEC model K, International Equipment Company, 300 Second Avenue, Needham Heights, MA 02194.

5.4 Balance, Mettler Analytical, model AE-240, analytical range 0-120 g, catalog number 01-909-407, Fisher Scientific, 711 Forbes Ave., Pittsburgh, PA 15219-4785.

5.5 Data System, model 59970, mass spectrometer, Hewlett Packard, 1601 California Ave., Palo Alto, CA 94304.

5.6 Gas Chromatograph, model 5890 Series II, Hewlett-Packard Avondale, PA.

5.7 Mass Selective Detector, Model 5971A, Hewlett-Packard, Palo Alto, CA.

5.8 Mechanical Shaker, model G-33, New Brunswick Scientific, catalog number 14-278-25, Fisher Scientific, Pittsburgh, PA.

5.9 Personal Computer, ChemStation, DOS series Software, model QS/120(386/25), operating on a Hewlett-Packard Vectra Personal Computer, Hewlett-Packard, Palo Alto, CA.

5.10 Vacuum Manifold, Visiprep, catalog number 5-7030, Supelco, Supelco Park, Bellefonte, PA 16823-0048.

5.11 Vacuum Pump, Sargeant-Welch, model 8803, catalog number 54969-828, capable of maintaining a minimum vacuum of 25 mm Hg, VWR, 4717 Hinckley Industrial Parkway, Cleveland, OH 44109.

5.12 Vial Crimper, catalog number 8710-0979, Hewlett-Packard Avondale, PA.

5.13 Vortex mixer, Genie 2, catalog number 12-812, Fisher Scientific, Pittsburgh, PA.

5.14 Water bath, Buchi, model B-461, catalog number 27559-641, VWR, Cleveland, OH.

6. Glassware(Note 15.1)

6.1 Bottle, clear glass, 8 oz. with Teflon®-lined screw cap, catalog number B7465-13, Baxter, 1430 Waukegan Road, McGaw Park, IL 60085-9988.

6.2 Column, capillary gas chromatography, Durabond-1(DB-1) bonded phase, 0.25 mm i.d. x 15 m, 0.25 µm film thickness, catalog number 122-1012, J & W Scientific, 91 Blue Ravine Road, Folsom, CA 95630-1899.

6.3 Column, pre-column, Stabilwax bonded phase, 0.25 mm x 1 m, 0.25 µm film thickness, catalog number 10623, Restek, 110 Benner Circle, Bellefonte, PA 16823-8812.

6.4 Inlet Liner, silanized dual tapered, splitless, catalog number 5181-3315, Hewlett-Packard, Avondale, PA.

6.5 Cylinder, graduated, 100 mL, with pour spout, catalog number C9085-100, Baxter, McGaw Park, IL.

6.6 Cylinder, graduated, 1L, with pour spout, catalog number C9085-1L, Baxter, McGaw Park, IL.

6.7 Flask, 2000 mL, Pyrex, Erlenmeyer with side-arm, to collect loading solutions, catalog number 0-180G, attaches to Supelco Vacuum Manifold, Fisher Scientific, Pittsburgh, PA.

6.8 Flask, volumetric, 10 mL, catalog number F4663-10A, Baxter, McGaw Park, IL.

6.9 Flask, volumetric, 50 mL, catalog number F4663-50A, Baxter, McGaw Park, IL.

6.10 Flask, volumetric, 100 mL, catalog number F4663-100A, Baxter, McGaw Park, IL.

6.11 Pipet, glass disposable, 1 mL, catalog number 13-678-25B, Fisher Scientific, Pittsburgh, PA.

6.12 Pipet, Pasteur, 2 mL, catalog number 13-678-20C, Fisher Scientific, Pittsburgh, PA.

6.13 Pipet, glass disposable, 5 mL, catalog number 13-678-25D, Fisher Scientific, Pittsburgh, PA.

6.14 Pipet, Glass disposable, 10 mL, catalog number 13-678-25E, Fisher Scientific, Pittsburgh, PA.

6.15 Pipet, glass, volumetric, 20 mL, catalog number 13-650N, Fisher Scientific, Pittsburgh, PA.

6.16 Pipet, glass disposable, 25 mL, catalog number 13-676-29D, Fisher Scientific, Pittsburgh, PA.

6.17 Pipet, motorized, 100 µL, catalog number E2-100, Rainin, Woburn, MA.

6.18 Pipet, motorized, 1.0 mL, catalog number E2-1000, Rainin, Woburn, MA.

6.19 Pipet, motorized, 2.5 mL, catalog number E2-2500, Rainin, Mack Road, Woburn, MA 01801-4628.

6.20 Tube, 15 mL conical, graduated in 0.1 mL increments, Pyrex 8082, catalog number C3980-15, Baxter, McGaw Park, IL.

6.21 Vials, autosampler, 2 mL, catalog number 1100, Sunbroker, P.O. Box 2230, Wilmington, NC 28402.

6.22 Vials, 20 mL, with Teflon®/silica septa caps, catalog number 2757FL, Industrial Glassware, P.O. Box 5, Millville, NJ 08332.

7. Materials(Note 15.1)

7.1 Adapters, SPE, Bakerbond, catalog number 7122-00, VWR, Cleveland, OH.

7.2 Caps, Tefion®/Silicon/Teflon® septum, crimp top, to fit autosampler vials, catalog number C4011-2A, National Scientific, 975 Progress Circle, Lawrenceville, GA, 30243.

7.3 Caps for 8 oz jar, catalog number B7465-13, Baxter, McGaw Park, IL.

7.4 Caps for the 20 mL vial, catalog number 2757FL, Industrial Glassware, Millville, NJ.

7.5 Caps, Teflon®-lined screw cap to fit 15 mL conical tubes, 17 mm, catalog number C3980-15, Baxter, McGaw Park, IL.

7.6 Cartridges, Bakerbond solid phase extraction (SPE), Octadecyl silyl, 1 g/6 mL size, catalog number 70 20-07, VWR, Cleveland, OH.

7.7 Gas, Helium, 99.999%, catalog number 801-M-26513, Air Products, 7201 Hamilton Blvd., Allentown, PA 18195.

7.8 Gas, Nitrogen, 99.998%, catalog number K01-3-39017, Air Products, Allentown. PA.

7.9 pH paper, Whatman CF strips, 0-14 pH range, catalog number 09-876-17, Fisher Scientific, Pittsburgh, PA.

7.10 Rack, Test Tube, S/P brand heavy duty polypropylene, 30 mm, catalog number S9262, Baxter, McGaw Park, IL.

7.11 Tubing, Teflon®, 2.1 mm i.d x 1/8" O.D., catalog number 2-0532M, Supelco, Bellefonte, PA.

8. Chemicals(Note 15.1)

8.1 Acetone, Burdick and Jackson, HPLC grade, catalog number 015-1DK, Baxter, McGaw Park, IL.

8.2 Acetonitrile, Burdick and Jackson, HPLC grade, catalog number 015-1DK, Baxter, McGaw Park, IL.

8.3 Acid, phosphoric, 85.0 %, Baker, Analyzed Reagent grade, catalog number 0260-01, VWR, Cleveland, OH.

8.4 BF_3-Methanol (12% w/w), catalog number 3-3040M, Supelco, Bellefonte, PA.

8.5 4-Chlorophenol, analytical standard, DowElanco, 9330 Zionsville Road, Indianapolis, IN, 46268-1053.

8.6 4-Chlorophenoxyacetic acid, analytical standard, DowElanco Indianapolis, IN.

8.7 4-Chlorophenoxyacetic acid methyl ester, analytical standard, DowElanco, Indianapolis, IN.

8.8 2,4-Dichlorophenoxyacetic acid 2-ethylhexyl ester, analytical standard, DowElanco, Indianapolis, IN.

8.9 2,4-Dichlorophenoxyacetic acid dimethylamine salt, analytical standard, DowElanco, Indianapolis, IN.

8.10 2,4-Dichlorophenoxyacetic acid, analytical standard, DowElanco, Indianapolis, IN.

8.11 2,4-Dichlorophenoxyacetic acid methyl ester, analytical standard, DowElanco, Indianapolis, IN.

8.12 2,4-Dichlorophenol, analytical standard, DowElanco, Indianapolis, IN.

8.13 2,4-Dichloroanisole, analytical standard, DowElanco, Indianapolis, IN.

8.14 Hexane, Burdick and Jackson, HPLC grade, catalog number 230-1DK, Baxter, McGaw Park, IL.

8.15 Methanol, Burdick and Jackson, HPLC grade, catalog number 230-1DK, Baxter, McGaw Park, IL.

8.16 PFTBA, 99.9%, for tuning the mass spectrometer, catalog number 8500-0656, Hewlett-Packard, Avondale, PA.

8.17 Toluene, Burdick and Jackson, Pesticide Residue grade, catalog number 347-1DK, Baxter, McGaw Park, IL.

8.18 Water, Distilled, Magnetic Springs, 1801 Lone Eagle Street, Columbus, OH 43228.

9. Reagents and Solutions

9.1 4% (v:v) acetone in hexane. Prepared by combining 4 mL acetone with 96 mL hexane.

9.2 10% (v:v) methanol in acetone. Prepared by combining 10 mL methanol with 90 mL acetone

9.3 15% phosphoric acid in water. Prepared by combining 30 mL concentrated (85 %) phosphoric acid with 143 mL distilled water.

9.4 0.15% phosphoric acid in water. Prepared by combining 10 mL of 15% phosphoric acid in water with 990 mL distilled water.

10 Preparation of Standards

10.1 Weigh out 0.1000 g 2,4-D 2-EHE analytical standard. Place it into a 100 mL volumetric flask. Rinse the weighing vessel with acetonitrile and transfer the rinsate to the volumetric flask. Dilute to the mark with acetonitrile to prepare an approximate 1000 µg/mL stock solution.

10.2 Weigh out 0.1000 g 2,4-D analytical standard. Place it into a 100 mL volumetric flask. Rinse the weighing vessel with acetonitrile and transfer the rinsate to the volumetric flask. Dilute to the mark with acetonitrile to prepare an approximate 1000 µg/mL stock solution.

10.3 Weigh out 0.1000 g 2,4-DCP analytical standard. Place it into a 100 mL volumetric flask. Rinse the weighing vessel with acetonitrile and transfer the rinsate to the volumetric flask. Dilute to the mark with acetonitrile to prepare an approximate 1000 µg/mL stock solution.

10.4 Weigh out 0.1000 g 2,4-DCA analytical standard. Place it into a 100 mL volumetric flask. Rinse the weighing vessel with acetonitrile and transfer the rinsate to the volumetric flask. Dilute to the mark with acetonitrile to prepare an approximate 1000 µg/mL stock solution.

10.5 Weigh out 0.1000 g 4-CP analytical standard. Place it into a 100 mL volumetric flask. Rinse the weighing vessel with acetonitrile and transfer the rinsate to the volumetric flask. Dilute to the mark with acetonitrile to prepare an approximate 1000 µg/mL stock solution.

10.6 Weigh out 0.1000 g 4-CPA analytical standard. Place it into a 100 mL volumetric flask. Rinse the weighing vessel with acetonitrile and transfer the rinsate to the volumetric flask. Dilute to the mark with acetonitrile to prepare an approximate 1000 µg/mL stock solution.

10.7 Weigh out 0.1195 g 2,4-D DMAS analytical standard. Place it into a 100 mL volumetric flask. Rinse the weighing vessel with acetonitrile and transfer the rinsate to the volumetric flask. Dilute to the mark with acetonitrile to prepare an approximate 1000 µg/mL acid equivalent stock solution.

10.8 Weigh out 0.1063 g 2,4-D methyl ester analytical standard. Place it into a 100 mL volumetric flask. Rinse the weighing vessel with acetonitrile and transfer the rinsate to the volumetric flask. Dilute to the mark with acetonitrile to prepare an approximate 1000 µg/mL acid equivalent stock solution.

10.9 Weigh out 0.1075 g of 4-CPA ME analytical standard. Place it into a 100 mL volumetric flask. Rinse the weighing vessel with acetonitrile and transfer the rinsate to the volumetric flask. Dilute to the mark with acetonitrile to prepare an approximate 1000 µg/mL acid equivalent stock solution.

10.10 Fortification Standards

10.10.1 Pipet 10 mL each of the stock standards 10.1-10.6 into a 100 mL volumetric flask and dilute to the mark with acetone to give a mixture containing 100 µg/mL of each standard.

10.10.2 Pipet 10 mL of solution 10.10.1 into a 100 mL volumetric flask and dilute to the mark with acetone to give a mixture containing 10 µg/mL of each standard.

10.10.3 Pipet 10 mL of solution 10.10.2 into a 100 mL volumetric flask and dilute to the mark with acetone to give a mixture containing 1 µg/mL of each standard.

10.10.4 Repeat steps 10.10.1 through 10.10.3 using stock standards 10.3 through 10.7.

10.10 5 Fortify 100 mL portions of water with the appropriate analytes(Note 15.2) by utilizing the appropriate aliquot(Column 2) of the appropriate standard solutions (Column 1) in the table below to obtain fortification concentrations ranging from 0.001 to 1.0 ppm(Column 3).

Column 1	Column 2	Column 3
Concentration of the Initial Solution	Aliquot of the Initial Solution	Fortification Level
µg/mL	mL	ppm
1	100	0.001
10	100	0.010
100	500	0.10
100	1000	1.0

10.11 Calibration Standards

10.11.1 Pipet 1 mL each of 10.1, 10.3, 10.4, 10.5, 10.8, and 10.9 into a 100 mL volumetric flask, add 10 mL acetone(Note 15.3), and dilute to volume using toluene to give a solution concentration of 10 µg/mL for each analyte.

10.11.2 Serially dilute the solution from 10.11.1 with toluene to obtain GC/MSD standards for 2,4-D 2-EHE, 2,4-D ME, 2,4-DCP, 2,4-DCA, 4-CP, and 4-CPA ME, as shown in the following table:

Concentration of Initial Solution	Aliquot of Initial Solution	Volume of Final Solution	Concentration of Final Solution
µg/mL	mL	mL	µg/mL
10.0	40	100	4.0
4.0	10	100	0.40*
0.4	25	50	0.20*
0.2	25	50	0.10*
0.4	10	100	0.04*
0.2	10	100	0.02*

*Use for GC standards covering a 20-fold concentration range with the 0.02 ug/mL standard being equivalent to 40 % of the LOQ.

11. Gas Chromatography/Mass Spectrometry

11.1 General

11.1.1 Install the splitless liner and the capillary column and pre-column on the split/splitless port of the GC/MSD following the manufacturer's recommended procedure.

11.2 Operating Conditions

11.2.1 To obtain optimum performance for the instrument, an autotune is conducted before the analysis of a set of samples. The autotune should be done at 170°C which is mid-range on the GC temperature program where most of the analytes will be eluting. The ions at m/z 69, 219, and 502 from perfluorotributylamine(PFTBA) are used to autotune the instrument. The autotune adjusts MS parameters and calibrates the mass axis so that the instrument will achieve maximum performance. Results from the autotune report should be compared on a daily basis to point out drifts or the need for ion source cleaning.

11.2.2 The analysis of the target analytes will be performed in the selected ion monitoring (SIM) mode. The ions to be monitored for each analyte are shown below:

Analyte	Quantitation Ion	Qualifier Ion 1	Qualifier Ion 2
2,4-D 2-EHE	222	332	220
2,4-D ME	234	236	199
2,4-DCP	162	164	98
2,4-DCA	176	161	178
4-CP	128	130	100
4-CPA ME	200	202	141

11.2.3 Typical operating conditions for the analysis of 2,4-D 2-EHE, 2,4-D as its methyl ester, 2,4-DCP, 2,4-DCA, 4-CP, and 4-CPA as its methyl ester are summarized in the table below:

Instrumentation	Hewlett-Packard model 5890 Series II gas chromatograph/model 5971A mass selective detector
Column	Durabond-1(DB-1), 0.25 mm i.d. x 15 m, 0.25 µm film thickness
Pre-Column	Stabilwax, 0.25 mm i.d. x 1 m, 0.25 µm film thickness
Oven Temperature	Hold at 60° for 2 minutes, then 60-150°C at a rate of 10°C/minute, then, 150-200°C at a rate of 45°C/minute, then, 200-240°C at a rate of 10°C/minute, then, hold at 240°C for 2 minutes
Injector Temperature	250°C
Transfer Line Temperature	280°C
Carrier Gas	Helium
Carrier Gas Flow Rate	~40-60 cm/second (internal flow sensor)
Head Pressure	5 psi
Injection Mode	Splitless
Injection Liner	Silanized dual taper
Injector Purge Delay	1.5 minutes
Septum Purge	~7.5 mL/minute
Injection Volume	2 µL
Ionization Potential	70 eV
Electron Multiplier Voltage	1400-1900 V (typical)
Dwell Time	200 msec

11.2.4 A typical total ion chromatogram of the six analytes is shown in Figure 1.

11.2.5 A mass spectrum for each analyte is shown in Figures 2 through 7.

12. Recovery of 2,4-D 2-EHE, 2,4-D DMAS as its Acid Equivalent, 2,4-D, 2,4-DCP, 2,4-DCA, 4-CP, and 4-CPA,

12.1 Place 100 mL of water sample into each of a series of 8 oz. screw-cap glass bottles.

12.2 Add 1 mL of 15% phosphoric acid, cap with a Teflon®-lined cap, and shake by inversion for 10 seconds to mix.

12.3 Use part of the samples as controls and fortify the remaining samples by adding 100 µL to 1000 µL of the appropriate fortification solutions to obtain analyte concentrations ranging from 0.001 to 1.0 ppm.

Treat each sample as follows

12.4 Thoroughly shake the bottles by hand for approximately 30 seconds.

12.5 Using pH paper, ensure that the pH of each sample is less than 2.

12.6 Condition 1 g, 6 mL, C_{18} Solid Phase Extraction cartridges by pulling through under vacuum, 10 mL of methanol, followed immediately by 10 mL of 0.15% phosphoric acid in water(Note 15.4). Do not allow cartridge to dry before loading sample.

12.7 Using a pasteur pipet, transfer a portion of each sample to its corresponding cartridge(Note 15.5).

12.8 Connect the SPE cartridge to the sample bottle using a SPE adapter(Figure 8).

12.9 Charge the acidified water samples onto the SPE cartridges at a flow rate of approximately 5 mL/min. using a vacuum manifold and discard the eluate(Note 15.6).

12.10 When the solution has completely passed through the SPE cartridge, allow each cartridge to dry under a vacuum of 20" Hg for a minimum of 20 minutes to remove excess water. Remove any remaining droplets adhered to the sides of the cartridge with a cotton swab or clean tissue. It is crucial that the water is removed or problems will occur during methylation.

12.11 Remove the cartridge set-up from the manifold and rinse the manifold ports with acetone to remove water.

12.12 After removing the SPE adapters and tubing, replace the SPE cartridges for subsequent elution of the analytes.

12.13 Elute the SPE cartridge under vacuum as follows:

12.13.1 Add 6 mL of 4% acetone in hexane to the sample bottle, then, cap and shake it. Transfer the solvent to the SPE tube with a Pasteur pipet. Elute the sample into a 15 mL conical centrifuge tube and designate it as FRACTION A.

12.13.2 Add 5 mL of 4% acetone in hexane. Collect into the same conical tube with FRACTION A(Note 15.7).

12.13.3 Add 5 mL of 10% methanol in acetone and collect the eluant in a 20 mL vial(6.22 above) and designate as FRACTION B(Note 15.8).

12.14 Under a gentle stream of nitrogen in an N-Evap(Note 15.9), evaporate FRACTION B to a final volume of 0.5 to 1.0 mL.

12.15 Add 1 mL of BF_3-methanol solution to each concentrated solution of Fraction B.

12.16 Cap tightly with a Teflon®-lined screw cap. Shake side-to-side once or twice to ensure complete mixing.

12.17 Immerse in a 70°C water bath for 30 minutes (Note 15.10). Ensure that the caps remain tight.

12.18 Cool the reaction mixture.

12.19 Add 8 mL of distilled water to each sample tube.

12.20 Add 5 mL of hexane to each sample and cap the tube.

12.21 Shake on a mechanical shaker for 10 minutes at approximately 180 excursions/minute.

12.22 Centrifuge the sample for about 3 minutes at approximately 2000 rpm.

12.23 Using a Pasteur pipet, remove the water layer and discard.

12.24 Using a Pasteur pipet, combine the hexane layer containing the methylated FRACTION B(Note 15.11) with the pooled FRACTION A into one solution.

12.25 Add 1 mL of toluene to the solution and shake briefly to ensure mixing.

12.26 Evaporate the solvents to a volume of slightly less than 1 mL under a gentle nitrogen stream in an N-Evap at a flow rate of approximately 25 mL/minute distributed through the stainless steel capillary tubes. Set the tubes so that the nitrogen initially impinges the surface of the solvent from a height of approximately 2 mm. As the solvent evaporates lower the tubes accordingly. Maintain the water bath at 20°C.

12.27 Bring to a final volume of 2.0 mL with toluene.

12.28 Vortex the sample on medium speed for 10 seconds to mix.

12.29 Transfer a portion of the concentrate to a GC vial and cap using a crimper(Note 15.12).

12.30 Inject 2 µL of the sample onto the GC/MSD to quantitate(Note 15.13).

12.31 Using toluene as the solvent, dilute any samples that are outside the 20 fold concentration range of the standard curve.

13. Determination/Calculation of Percent Recovery

13.1 For analytes 2,4-D 2-EHE, 2,4-D ME, 2,4-DCP, 2,4-DCA, 4-CP, and 4-CPA ME:

13.1.1 [illegible] e calibration standards described in section [illegible] etermine the peak areas of the appropriate [illegible] n ions.

[illegible] a standard curve by plotting the quantitation peak area versus the calibration standard concentrations.

13.1.3 Determine the concentration in µg/mL of the analytes found in the recovery samples by comparing their peak areas to the standard curve.

13.1.4 Calculate the concentration in the final solution as follows:

$$\mu g/mL = \frac{peak\ area - C}{m}$$

where, m is the slope and C is the y-intercept of the regression line obtained from a plot of the peak area versus the nominal standard concentration.

13.1.5 The analyte concentration in the original water sample (in ppm) is calculated using the equation shown below:

$$ppm = \text{ug/mL in final solution} \times 0.02 \times \text{any additional dilution}$$

13.1.6 Positive confirmation of the presence of each analyte is indicated when the confirmation ion ratio is within ±20% of the average ion ratio found for the standards.

Calculate the confirmation ion ratio using the equation shown below:

$$\text{Confirmation ratio} = \frac{\text{peak area Qualifier 1}}{\text{peak area Quantitation Ion}}$$

13.1.7 Subtract any contribution from the control sample to obtain the corrected ppm, then, calculate the percent recovery as shown below:

$$\text{Recovery(\%)} = \frac{\text{Corrected ppm}}{\text{ppm added}} \times 100$$

14. Determination of 2,4-D 2-EHE, 2,4-D, 2,4-DCP, 2,4-DCA, 4-CP, and 4-CPA in Water

14.1 Prepare treated samples as described in Section 12., steps 12.1 through 12.30, omitting the fortifications in step 12.3.

14.2 Determine the concentration in µg/mL from the standard curves. Typical standard curves are shown in Figures 9 through 14.

14.3 Calculate the µg/mL in the final solution as described in section 13.1.4.

14.4 Calculate the original water concentration using the equation shown below:

ppm = ug/mL in final solution x 0.02 x any additional dilution

14.5 Typical total ion chromatograms of a control water and control water fortified at 0.001 ppm are shown in Figures 15 and 16.

Typical Selected Ion Monitoring chromatograms for each analyte are shown in Figures 17 and 18.

14.6 Recoveries for each analyte over the concnetration range 0.001 to 1.0 ppm in water averaged 88, 112, 94, 94, 105, and 95%. for 2,4-DCA, 2,4-DCP, 4-CPA, 4-CP, 2,4-D, and 2,4-D 2-EHE, respectively. The results are summarized in Table 1.

14.7 A typical analytical set could consist of two sets of twelve analyses made up of any combination of reagent blank(s), controls, fortified controls, and field samples. These twenty four analytical samples can be carried through to just before methylation in one eight hour day. The twenty four analytical samples can be carried through to quantitation in one ten hour day.

14.8 Two convenient stopping points relative to volatilization and degradation are step 12.14 just before methylation and step 12.29 after encapsulation. In all cases the samples should be refrigerated when stored.

15. Notes

15.1 Equipment, glassware, materials, reagents, and chemicals considered to be equivalent to those specified may be substituted with the understanding that their performance must be confirmed by appropriate tests

15.2 The fortification solution containing 2,4-D 2-EHE is used for sample sets in which 2,4-D 2-EHE is under investigation. The solution containing 2,4-D DMAS is used for sample sets in which 2,4-D DMAS is under investigation

15.3 Toluene and acetonitrile are immiscible so acetone is added to obtain a single phase, uniform solution.

15.4 Condition cartridges at a vacuum level less than 5 in. Hg or a flow rate of 1 mL/minute. Do not allow the vacuum to exceed 5 in. Hg or a flow rate of approximately 1-2 mL/minute.

15.5 Run Teflon® tubing from the SPE adapter to the sample bottle. The sample bottles must be raised above the level of the vacuum manifold in order to more efficiently pull the entire sample through the SPE cartridge.

15.6 Vacuum should be maintained at < 10" of Hg.

15.7 This solution contains 2,4-D 2-EHE, 2,4-DCP, 2,4-DCA, and 4-CP. These analytes are chromatographed without derivatization.

15.8 This solution contains 2,4-D and 4-CPA which are derivatized before analysis.

15.9 Use a Meyer N-Evap with capillary stainless steel tubing and a flow rate of 10 to 25 mL/minute.

15.10 The water bath should be set at a temperature of 70 ± 2°C for the methylation reaction. Immerse the tube so that the water level is just above the level of the solution in the tube.

15.11 This solution contains the methyl esters of 2,4-D and 4-CPA.

15.12 All analytes are chromatographed on the same gas chromatography system using the same conditions.

15.13 2,4-D 2-EHE is only monitored for if it is one of the analytes under investigation. 2,4-D DMAS is determined as its methyl ester.

15.14 2,4-D and 4-CPA concentrations can be determined directly from the quantities of 2,4-D methyl ester and 4-CPA methyl ester, respectively, because the calibration solutions used to generate the 2,4-D methyl ester and 4-CPA methyl ester calibration curves are prepared by taking into account the difference in molecular weight between the acids and their methyl esters. For example, the "1000 µg/mL" solution of 2,4-D methyl ester prepared in step 10.8 actually has a concentration of 1063 µg/mL. This concentration is equivalent to 1000 µg/mL of 2,4-D. The 1063 µg/mL solution is used to prepare the 2,4-D methyl ester calibration standards with acid equivalent concentration of 0.02 ppm, 0.04 ppm, etc. and, therefore, any 2,4-D methy ester peaks detected can be converted to 2,4-D concentrations directly.

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WATER METHOD MODIFICATIONS
(RAM 8862-93-002, Revision 01 (3/1/93))

Number	Step(s) Modified	Description	Reason	Impact on Study
1	11.2.1	A manual tune was performed as opposed to using the autotune software.	The autotune software, version 1.12, was unable to yield consistent and satisfactory tuning results. The manual tune was performed and printed to show consistency with previous tuning parameters. If this was not possible, parameters were changed or the source was cleaned until successful.	Improved consistency throughout study.
2	11.2.3	A DB-5MS (30m x 0.25 mm, 0.5µ) column was substituted & no pre-column was used.	Cleanliness of matrix allowed the use of a slightly higher polarity column.	Improved chromato-graphy.
3	11.2.3	The initial oven temperature was increased from 60°C initially to 90°C. The final oven temperature was increased from 240°C to 280°C. The steps in the ramp were similar relative to the new initial and final temperatures.	The use of toluene necessitated a higher initial temperature to avoid trapping the sample at the head of the column.	Increased life of injection liner and the head of column.
4	12.5	pH meter was used instead of pH paper.	The difference in the amount of acid needed to reach pH 2 seemed to be variable and the paper was not highly accurate.	Increased extraction efficiency by ensuring pH was 2 or less.

WATER METHOD MODIFICATIONS (Continued)

Number	Step(s) Modified	Description	Reason	Impact on Study
5	12.13.1	Fraction A solvent was changed from 4% acetone in hexane to 1.5% acetone in hexane.	To improve the separation between analytes. The lower the concentration of acetone in hexane, the longer the 2,4-D & 4-CPA remain on the column leading to increased recovery.	Improved recovery of 2,4-D and 4-CPA.
6	12.13.3	Fraction B was collected in a 15-mL culture tube rather than a 20-mL vial.	The culture tube, as opposed to the vial, allowed for a certain amount of reflux during derivitization.	Increased recovery of 2,4-D and 4-CPA.
7	12.14 & 12.26	A manifold was used for evaporation rather than an N-Evap.	An N-Evap was not available.	None
8	12.19	Derivitization was performed in a culture tube and transferred to a 20-mL vial for hexane partition.	The vial provided a better partition than the culture tube due to the diameter of each.	Improved removal of any polar contaminants
9	12.22	Centrifugation was not performed.	No emulsion was present. The hexane and water seemed to separate quite well without centrifugation.	None