Chlorophenoxy acids, total recoverable (0-3105-83) and dissolved (0-1105-83), gas chromatographic

	Code	
Parameter	*Total recoverable*	*Dissolved*
2,4-D	39730	39732
2,4-DP	82183	82356
Silvex	39760	39762
2,4,5-T	39740	39742

1. Application

This method is suitable for the determination of chlorophenoxy acid herbicides, and their esters and salts, in water and water-suspended-sediment mixtures containing at least 0.01 µg/L of the analyte.

2. Summary of method

Chlorophenoxy acid herbicides and their esters are extracted with either diethyl or methyl t-butyl ether from an acidified water sample. The extracted herbicides are hydrolyzed to the free acids which are converted to their methyl esters with boron trifluoride-methanol and purified using adsorption chromatography. The methyl esters are determined by gas chromatography using electron capture detectors.

3. Interferences

Halogenated organic acids, and their salts and esters, may cause interference.

4. Apparatus

4.1 *Boiling chips,* granules, micro, Hengar, H-1366C, or equivalent: Rinse with hexane, air dry, and heat at 300°C overnight.

4.2 *Centrifuge tube,* 5 mL, Pyrex, graduated, with ground-glass stopper.

4.3 *Concentrator, Kuderna-Danish (K-D),* 125-mL flask and 5.0-mL receiver, one-ball Snyder column, and modified micro-Snyder column, Kontes 569251, or equivalent.

4.4 *Florisil column,* a disposable glass pipet with glass-wool plug: Fill to a depth of 1.5 cm with florisil adsorbent, followed by 2 cm sodium sulfate.

4.5 *Gas chromatograph,* Tracor Model 550, or equivalent.

4.5.1 The following conditions are recommended:

Columns, borosilicate glass, 1.8 m x 2 mm id (inside diameter), operated at 180°C: Column packing materials are (1) 3 percent SP 2100 on 100/120 mesh Supelcoport; and (2) 3 percent SP 2250 on 100/120 mesh Supelcoport, or equivalent.

Detector, dual electron capture operated at 350°C.

Injection port temperature, 200 ° C.

Carrier gas, nitrogen, flow rate 20 mL/min.

4.6 *Glass filters,* 142 mm, 0.3 µm mean pore size, Gelman, or equivalent: Prepare the filters by rinsing with acetone and hexane, evaporating the solvent, and heating overnight at 300°C.

4.7 *Glass wool,* fine, rinsed with hexane, air dried, and heated at 300°C overnight.

4.8 *Sandbath,* Tecam, or equivalent.

5. Reagents

5.1 *Boron trifluoride-methanol esterification reagent,* 14 percent BF_3; (weight/volume; w/v) in methanol, Applied Science Labs, or equivalent.

5.2 *Florisil adsorbent,* commercially activated at 650°C, washed with hexane, allowed to air dry, and stored at 130°C in a glass-stoppered flask: Prior to use, the florisil is deactivated by adding 10 percent water by weight and shaking for at least 2 h on a wrist-action shaker. The florisil is then tested for activity by attempting to elute the herbicides of interest with benzene from a test column. If the test compounds do not elute within 2.0 mL, further deactivation is required until the desired results are obtained.

5.3 *Potassium hydroxide solution,* 37 percent (w/v): Dissolve 78 g KOH reagent-grade pellets in 200 mL deionized water. Reflux for 8 h.

5.4 *Sodium sulfate, acidified:* Prepare a slurry of sodium sulfate with enough diethyl ether to cover the crystals, and acidify to pH 2 or less by adding a few milliliters of concentrated sulfuric acid. Determine the pH by transferring a small portion of the slurry to a beaker and removing the ether by evaporation. Add a few drops of deionized water to the crystals and measure the pH with pH paper. Allow to air dry overnight. Store in a covered Pyrex beaker or flask at 130°C.

5.5 *Sodium sulfate, granular, anhydrous:* Heat overnight at 300°C and store covered at 130°C.

5.6 *Sodium sulfate solution,* 5 percent (w/v): Dissolve 50 g neutral sodium sulfate in deionized water and dilute to 1 L.

5.7 *Solvents,* benzene, unpreserved diethyl ether or methyl t-butyl ether, and iso-octane, distilled in glass, pesticide analysis quality, Burdick and Jackson, or equivalent: Diethyl ether preserved with ethanol cannot be used in this procedure because it results in the formation of extraneous ethyl esters.

5.8 *Standards, methyl esters of chlorophenoxy acid herbicides,* EPA analytical reference grade or equivalent: Prepare a stock solution by weighing about 10 mg of compound to at least three significant figures and transfer to a 25-mL volumetric flask. Dilute to volume with benzene and mix thoroughly. Prepare a series of mixed-compound standards by volumetric dilution with iso-octane, as described in table 1.

5.9 *Sulfuric acid,* concentrated (sp. gr. 1.84), Mallinckrodt analytical reagent, A.C.S. grade, or equivalent.

5.10 *Sulfuric acid,* (1 + 3): Prepare by adding 1 part concentrated sulfuric acid to 3 parts deionized water. Store in a refrigerator at 4°C.

5.11 *Water,* deionized, organic-free.

6. Procedure

All glassware must be washed in warm detergent solution, rinsed with organic-free water, and heated at 300°C overnight. Prior to use, all glassware is rinsed with the solvent it will contact. Stopcock grease should not be used on ground-glass joints.

Table 1. Concentration of herbicides in mixed standard solutions used for gas chromatograph calibration of water and water-suspended sediment

[Picograms per microliter]

Herbicide	High standard concentration	Low standard concentration
2,4-DP	100	50
2,4-D	100	50
2,4,5-T	40	20
Silvex	40	20

For the determination of dissolved components, filter the sample through a glass filter to remove the suspended material. Pour the filtrate into the original sample bottle and continue with the procedure.

6.1 Immediately upon receipt of a sample in the laboratory, it must be acidified to pH 2 or lower with concentrated sulfuric acid and stored at 4°C.

6.2 A blank must accompany each group of samples. For each sample, rinse a 1,000-mL separatory funnel and a 250-mL Erlenmeyer flask with ether.

6.3 Weigh the sample bottle plus sample and record the weight to three significant figures.

6.4 Pour the sample into the separatory funnel and allow the bottle to drain completely. Weigh the empty bottle and record the weight to three significant figures. Calculate and record the sample weight.

6.5 Add 150 mL ether to the sample bottle, rinse the sides thoroughly, and pour the solvent into the separatory funnel. The Teflon-lined cap is not rinsed because of the potential for contamination from solvent that has contacted the threads and the surface beneath the Teflon liner. Shake the funnel vigorously for at least 1 min, venting often. Allow the layers to separate and drain the aqueous layer. Pour the ether extract into the Erlenmeyer flask. Extract the sample twice more, using 50 mL ether each time, and collect the extracts in the Erlenmeyer flask.

6.6 Add 15 mL distilled water, 2.0 mL 37 percent KOH, and a boiling chip to the extract. Fit the flask with a Snyder column and heat the assembly on a steam bath for a total of 90 min, during which time the ether will evaporate and the herbicide esters are hydrolyzed.

6.7 Remove the assembly from the water bath, allow to cool, and quantitatively transfer the water to a 125-mL separatory funnel. Extract the basic solution with 20 mL ether and discard the ether layer; repeat twice with 10 mL ether and discard the ether layers. The herbicide potassium salts remain in the aqueous phase. Add 2 mL sulfuric acid (1+ 3) to the contents of the funnel to bring the pH to 2 or below, and extract the aqueous phase with 20 mL ether; repeat twice with 10 mL ether to extract the herbicides in their acid forms. Collect the ether extracts in a 125-mL Erlenmeyer flask containing about 0.5 g acidified sodium sulfate. Cover the flask with foil and set aside for at least 1 h, or store in a refrigerator until analysis can continue.

6.8 Quantitatively transfer the ether extract into a K-D apparatus fitted with a 5-mL volumetric receiver. Add 1 mL benzene and a boiling chip. Concentrate the extract to about 0.5 mL on a fluidized sandbath heated to 60-70°C. Under no circumstances should the extract be allowed to evaporate completely to dryness. Clear sand from the glass joint before opening. (Use a water bath at 80°C for methyl t-butyl ether extracts.) Rinse the bottom joint with benzene into the receiver.

6.9 After the benzene solution in the receiver has cooled, add 0.5 mL boron trifluoride-methanol reagent. The modified Snyder column is used as an air-cooled condenser, and the contents of the receiver are held at 50°C for 30 min in a sandbath. Cool the reaction mixture to room temperature and add sodium sulfate solution until the benzene-aqueous solution interface is observed in the restricted neck of the receiver. Stopper the receiver, shake vigorously for 1 min, and allow to stand for at least 1 h for phase separation. Loosen the stopper after shaking.

6.10 Transfer the benzene layer from the receiver to a florisil column. Elute with benzene until a total of 2.0 mL of benzene has been collected in a graduated centrifuge tube. Analyze the eluate by gas chromatography.

6.11 Prepare gas chromatograph calibration curves daily with the mixed standards listed in table 1. Operating conditions must be identical to those used for sample analysis. Calibrate both analytical columns. Record the volume of the standard injected and the retention time and integrated peak area of each component in the standard.

6.12 Inject an aliquot of sample extract into the gas chromatograph. Record the volume injected. Identify peaks by retention time. The identification must be made on both analytical columns. Record the retention time and integrated area of any identified peak. Dilute any extract containing an identifiable component above the highest standard.

7. Calculations

7.1 Calculate the response factor of each identified component in the calibration standard:

$$RF = \frac{A_1}{C_s \times V_1}$$

where

RF= response factor of identified component in calibration standard, in area/pg,

C_8 = concentration of standard, in pg/ µL, (step 5.8),

V_1 = volume of standard injected, in µL (step 6.11), and

A_1 = integrated peak area of an identified component in calibration standard (step 6.11).

7.2 Calculate the concentration of each identified component in the original sample from the equation

$$\text{Concentration } (\mu g/L) = \frac{A_2 \times V_2}{V_3 \times W \times RF},$$

where

RF= response factor of identified component in sample, in area/pg,

A_2 = integrated peak area of identified component,

V_2 = final volume of sample extract, in mL,

V_3 = volume of sample extract injected, in µL, and

W = weight of sample in g, expressed in mL (1.000 mL= 1.000 g).

7.3 Calculate the free chlorophenoxy acid concentration:

$$\text{Concentration of acid } (\mu g/L) = C \times f,$$

where

C = concentration of methyl ester (calculation 7.2), and

$$f = \frac{\text{molecular weight of acid}}{\text{molecular weight of methyl ester}}$$

8. Report

Report chlorophenoxy acid herbicide concentrations as follows: less than 0.01 µg/L, as "less than 0.01 µg/L"; 0.01 µg/L to 0.10 µg/L, one significant figure; 0.10 µg/L and above, two significant figures.

9. Precision

9.1 Precision for dissolved chlorophenoxy acids in deionized water for 35 replicates using diethyl ether as follows:

Compound	Spiked Concentration (µg/L)	Mean concentration determined (µg/L)	Relative standard deviation (percent)
2,4-D---------------	0.10	0.075	10.0
Silvex --------------	.048	.036	11.6
2,4,5-T-------------	.058	.045	12.2

9.2 Precision for dissolved chlorophenoxy acids in deionized water for 35 replicates using methyl t-butyl ether is as follows:

Compound	Spiked Concentration (µg/L)	Mean concentration determined (µg/L)	Relative standard deviation (percent)
2,4-D---------------	0.10	0.083	10.1
Silvex --------------	.048	.040	10.4
2,4,5-T-------------	.056	.049	10.0

9.3 It is estimated that the percent relative standard deviation for total recoverable chlorophenoxy acids will be greater than that reported for dissolved chlorophenoxy acids.

Selected references

Goerlitz, D.F., and Brown, Eugene, 1972, Methods for analysis of organic substances in water. U.S. Geological Survey Techniques of Water-Resources Investigations, Book 5, Chapter A3, 40 p.

Goerlitz, D.F., and Lamar, W.L., 1967, Determination of phenoxy acid herbicides in water by electron-capture and microcoulometric gas chromatography: U.S. Geological Survey Water Supply Paper 1817-C, 21 p.