Designation: D 5659 – 95 (Reapproved 2001)

Standard Test Method for Chlorophenoxy Acid Herbicides in Waste Using HPLC[1]

This standard is issued under the fixed designation D 5659; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers the analysis of 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and 2,4,5-trichlorophenoxypropionic acid (silvex) in liquids and solids, using high performance liquid chromatography with an ultraviolet detector (HPLC/UV). This test method is applicable for a concentration range from approximately 50 to 1000 ppm. This range takes into consideration the sample preparation and dilutions outlined in Section 10. Lower detection levels can be obtained by using larger sample sizes, smaller total final volumes, or with the use of in-line or solid phase extraction, concentration, and/or cleanup.

1.2 The chlorophenoxy herbicides may be present as a variety of salts or esters, which are converted to, analyzed, and reported as their respective acids.

1.3 This test method is applicable to liquid and solid waste and waste extract matrices including aqueous, oil, spent solvent, soil, ash, leachates, etc.

1.4 This test method may be applicable to other phenoxy acid herbicides.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* See Section 7 and 10.3.1 for specific precautionary statements.

2. Referenced Documents

2.1 *ASTM Standards:*

D 1193 Specification for Reagent Water[2]

D 3478 Test Method for Chlorinated Phenoxy Acid Herbicides in Water[3]

2.2 *EPA Documents:*

Method 8150 Chlorinated Herbicides, Test Methods for Evaluating Solid Waste Physical/Chemical Methods SW-846 Third Edition[4]

Method 8000A Gas Chromatography, Test Methods for Evaluating Solid Waste Physical/Chemical Methods, SW-846, 3rd Edition, Final Update 1[4]

Method 555 Determination of Chlorinated Acids in Water by High Performance Liquid Chromatography with a Photodiode Array Ultraviolet Detector EPA/600/R-92/129, Methods for the Determination of Organic Compounds in Drinking Water, Supplement No. 2[4]

3. Summary of Test Method

3.1 The chlorophenoxy acids and esters are hydrolyzed to their respective salts by heating and stirring the sample with aqueous alkali. The salts are then converted to their respective acids by the addition of HCl. The aqueous solutions of the free acids are then analyzed using High Performance Liquid Chromatography (HPLC) using ultraviolet detection.

4. Significance of Use

4.1 Phenoxy acid herbicides are used extensively for weed control. Esters and salts of 2,4-D, 2,4,5-T, and Silvex have been used for agricultural crop and lawn care.

5. Interferences

5.1 Organic liquids that have high percent levels of chlorinated organics and are denser than water may interfere with the extraction of Silvex. It is necessary to mix these samples with hexadecane, typically in a 1:1 ratio, before hydrolysis.

5.2 Phenols, especially chlorophenols interfere with the procedure, by coeluting with the analytes of interest.

5.3 Interferences may be encountered from other organic compounds that absorb UV at the specified wavelengths. Also, closely eluting compounds may complicate identification based solely on retention time. When these types of interferences are encountered, the analyst must rely on other sources of information for positive identification, such as the following:

5.3.1 Secondary confirmation wavelengths such as 227 nm or 235 nm.

5.3.2 Use of a confirmation column.

5.3.3 Use of a confirmatory chromatography program such as changing the mobile phase composition or gradient.

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[1] This test method is under the jurisdiction of ASTM Committee D34 on Waste Management and is the direct responsibility of Subcommittee D34.01.06 on Analytical Methods.

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[2] *Annual Book of ASTM Standards*, Vol 11.01.

[3] Discontinued, 1992. See *Annual Book of ASTM Standards*, Vol 11.02.

[4] Available from the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402.

6. Apparatus

6.1 *Analytical High-Pressure Liquid Chromatograph*, capable of achieving pressures of 4000 psi and flow rates of 3 mL/min.

6.2 *Variable Wavelength Ultraviolet Detector*, capable of monitoring at 207, 227, and 235 nm, either simultaneously or individually.

6.3 *Chromatographic Column*, C18 radial compression 8 by 100 mm, 4-µm particle size. Equivalent stainless steel or radial compression columns may be used.

6.4 *Guard Column*, C18, 4-µm particle size.

6.5 *Injector*, manual injection valve, instrument autosampler, equipped with a 500-µL sample loop, or equivalent.

6.6 *Data Systems*, data systems capable of controlling the HPLC system and for acquiring data may be used.

6.7 *Glass Vials*, 16-mL capacity with TFE-fluorocarbon-lined screw caps.

6.8 *Microsyringes*, 10, 100, and 500-µL capacity.

6.9 *Balance*, analytical, capable of accurately weighing to the nearest 0.0001 g.

6.10 *Pipets*, Pasteur, disposable glass.

6.11 *Pipets*, disposable glass, 1-mL and 10-mL, calibrated.

6.12 *pH paper*, wide range from 1 to 11.

6.13 *Hot Plate*, with multiple stirring positions.

6.14 *Water Filtration Apparatus*, used for the purification of water for HPLC use in 7.4.

6.15 *Water Filtration Filters*, 0.22-µm used in 7.4.

6.16 *Flasks*, 100-mL volumetric glass.

6.17 *Centrifuge*.

6.18 *Stir Bars*.

6.19 *Funnels*, glass.

6.20 *Filter Papers*, 15-cm hardened/ashless, fast.

7. Reagents and Materials

7.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society where such specifications are available.[5] Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water as defined by Type II of Specification D 1193.

7.3 *Acetone*, pesticide quality or equivalent, used for preparing standards. Be advised that if methanol is used for preparing the acid standards, over time the acid form of the herbicides will convert to their methyl ester form.

7.4 *Filtered Water with 0.5 % Phosphoric Acid*, (FWPA). Add 5 mL high-purity phosphoric acid to 995-mL reagent water in a volumetric flask. Filter through a 0.22-µm filter.

7.5 *Herbicide Reference Standards*—The following reference calibration compounds are required. Reference solutions can be prepared from the pure standard materials or purchased as certified solutions.

7.5.1 2,4-D, 2,4,5-T, Silvex, 2,4-D methyl ester.

7.5.2 2,4,5-T methyl ester, Silvex methyl ester.

7.6 *Hexadecane*, 99 %.

7.7 *Hydrochloric Acid* (density 1.195 g/mL), concentrated hydrochloric acid (HCl).

7.8 *Methanol*, pesticide quality or equivalent.

7.9 *Acetonitrile*, pesticide quality or equivalent. Acetonitrile may be used as the mobile phase instead of methanol.

7.10 *Phosphoric Acid*, H_3PO_4, 85 % o-phosphoric acid, HPLC Grade.

7.11 *Potassium Hydroxide 37 % Solution*—carefully add 37 g of ACS grade potassium hydroxide (KOH) pellets to 50 mL of reagent water in a 100-mL volumetric flask. Mix by swirling the flask. Bring to volume with additional reagent water.

NOTE 1—**Caution:** Observe caution when adding KOH to water. This will cause an exothermic reaction. Cooling the flask while mixing is suggested.

8. Standard Preparation

8.1 Prepare stock standard solutions as follows. For herbicide acids and methyl esters, accurately weigh 0.100 g into a 10-mL glass volumetric flask. Bring to volume in pesticide-grade acetone. Larger volumes can be used at the convenience of the analyst. If compound purity is 96 % or greater, the weight can be used without correction to calculate the concentration of the stock standard.

8.2 Transfer the stock standard solutions into TFE-fluorocarbon-sealed screw-cap vials. Store at 4°C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation especially prior to being used to prepare calibration standards. Marking the meniscus level on the standard vial is recommended when monitoring for evaporation.

8.3 Stock standard solutions must be replaced after one year or sooner if comparison with check standard indicates a problem.

8.4 Prepare calibration standard solutions from stock standard solutions at a minimum of 5 concentration levels for each parameter. Transfer the appropriate volume of stock solution to a volumetric flask and dilute with FWPA. The concentration levels should correspond to the expected range of concentrations found in real samples or should define the working range of the HPLC/UV. A working range from 0.5 to 5 ppm has been found to be useful. Calibration solutions must be replaced after six months, or sooner if comparison with check standards indicates a problem. A 20 % deviation in response between the daily check and the initial calibration would indicate a potential problem.

9. Sample Collection, Preservation, and Handling

9.1 Sample collection should be in accordance with appropriate sampling protocols.

9.2 Sample extracts should be stored in glass containers. Long-term storage should be at 4°C. For an aqueous matrix, 100 mL of sample are needed and 5 g of sample are needed for

[5] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K. and the *United States Pharmacopeia and National Formulary*, U.S. Pharmaceutical Convention, Inc. (USPC), Rockville, MD.

solids or organic liquids.

9.3 Unused sample material, laboratory dilutions, and waste from the samples may be regulated. Consult your specialist and the regulations, or both, for guidance in the proper handling and disposal of laboratory waste.

10. Procedure

10.1 *Sample Extraction and Hydrolysis (for Aqueous Matrices)*:

10.1.1 Add 50 mL or 50 g of sample to a 100-mL volumetric or Erlenmeyer flask.

10.1.2 Add 10 mL of 37 % KOH solution to the flask. Check the pH. If the pH is not 10 or greater, add additional KOH solution. Record the KOH solution used, in millilitres, and the final pH.

10.1.3 Add a stir bar to the flask. Place the flask on a stirring hot plate and heat and mix at 70 to 90°C for 2 h.

10.1.4 Continue to 10.3.

10.2 *Sample Extraction and Hydrolysis (All Other Matrices)*:

10.2.1 Weigh 5 g of sample into a 100-mL volumetric or Erlenmeyer flask.

NOTE 2—Be cognizant of sample matrix type, especially for solid materials such as soils and ash. It may be necessary to crush or powder the sample to ensure complete extraction.

10.2.1.1 If the sample is a dense organic liquid for example, high PCB oils, chlorinated solvents, and so forth, weigh 2.5 g of sample into a 100-mL volumetric flask and add 2.5 g hexadecane. Mix by swirling the flask.

NOTE 3—It was determined through recovery studies that the chlorophenoxy herbicides remained in highly chlorinated organic liquids on the bottom of the volumetric flask after hydrolysis. Mixing the sample with hexadecane brought the sample to the top of the volumetric flask and the herbicides were extracted into and remained in the aqueous phase after hydrolysis.

10.2.1.2 Add 50 mL reagent water to the flask. Mix by swirling the flask.

10.2.1.3 Add 10 mL of 37 % KOH solution to the flask. Check the pH. If the pH is not 10 or greater add additional KOH solution. Record millilitres of KOH solution used and the final pH.

10.2.1.4 Add a stir bar to the flask. Place the flask on a stirring hot plate and heat and mix at 70 to 90°C for 2 h.

10.2.1.5 For oil samples, after stirring is complete, remove stir bar and bring volumetric flask to volume with reagent water. The water meniscus should be at the mark. Let stand until the layers separate.

10.2.1.6 Draw off and discard any oil layer that may be present on top of the water.

10.2.1.7 For solid samples, filter extract through filter paper (see 6.20) into a separate 100-mL volumetric flask. Rinse the filter paper being careful not to exceed volume. Bring to volume with reagent water.

10.2.1.8 Continue to 10.4.

NOTE 4—This is a good stopping point in the method if needed. Store sample extracts overnight in the refrigerator.

10.3 *Preparation for Analysis (Aqueous Matrices)*:

10.3.1 Add 5 mL of concentrated HCl to the volumetric flask containing the solution prepared in 10.1. Mix well. **Caution: always wear personal protective gear when adding concentrated acid to water because of the potential for heat generation.**

10.3.2 Check the pH using wide-range pH paper. The pH must be less than two before HPLC analysis.

10.3.3 If the pH is not less than two, add additional HCl to the flask. Check and record the final pH.

10.3.4 Bring the volumetric flask to volume with reagent water.

10.4 *Preparation for Analysis (All Other Matrices)*:

10.4.1 Add 8 mL of FWPA prepared in 7.4, 1 mL concentrated HCl, and 1 mL of the sample extract prepared in 10.2 to a 16-mL glass vial. Shake.

10.4.2 Check the pH using wide-range pH paper. The pH must be less than two before HPLC analysis.

10.4.3 If the pH is not less than two, re-prepare the sample as in 10.4.1 using 7 mL FWPA and 2 mL concentrated HCl. Check and record the final pH.

10.4.4 If visible suspended solids are present in the sample, centrifuge the sample before injecting into the HPLC. It is very important to limit the amount of suspended solids injected onto the column and into the HPLC system because of the nature of HPLC and the potential for problems.

10.5 *HPLC Calibration*:

10.5.1 Using the calibration standards prepared in 8.4, generate a calibration curve that defines the working range or range of interest of the HPLC system. A correlation coefficient of 0.995 with a percent RSD of less than 20 is recommended for the acceptance of a valid calibration curve.

10.5.2 Check the initial calibration curve daily by injecting a mid-level standard. If the response varies from the initial calibration by more than 20 %, recalibration is recommended. If the daily standard and initial calibration is acceptable, use the response of the daily standard for H_{std} value in Section 11.

10.6 *HPLC Analysis*—Inject the solution prepared in 10.3 or 10.4 into the HPLC. A typical chromatogram and UV spectra are shown in Figs. 1 and 2.

10.7 *HPLC Conditions*:

10.7.1 *Mobile Phase*—75 % methanol/25 % FWPA.

NOTE 5—Optimum separation may require different percentages of organic and aqueous constituents if acetonitrile is used as the mobile phase in place of methanol.

10.7.2 *Flow Rate*—2.5 mL/min.

10.7.3 *Detector Wavelength*—primary quantitation at 207 nm.

10.8 *Quality Control*:

10.8.1 It is recommended that each laboratory using this test method operate a formal quality control program. The minimum requirements of this program should consist of an initial demonstration of laboratory capability and the on-going analysis of method blanks, duplicate and spiked samples to evaluate and document quality data.

10.8.2 Before processing any samples, the analyst must demonstrate, through the analysis of a method blank, that the interferences from the analytical system, glassware, and reagents are under control. Each time a set of samples is prepared or there is a change in reagents, a method blank must be

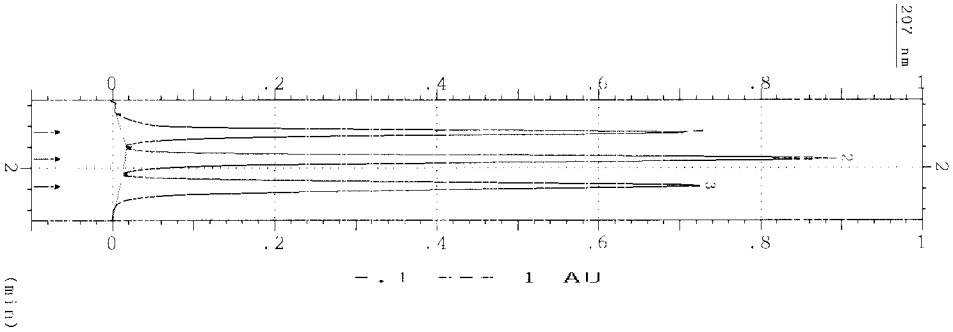

FIG. 1 Typical Chromatogram Using Conditions in 10.7

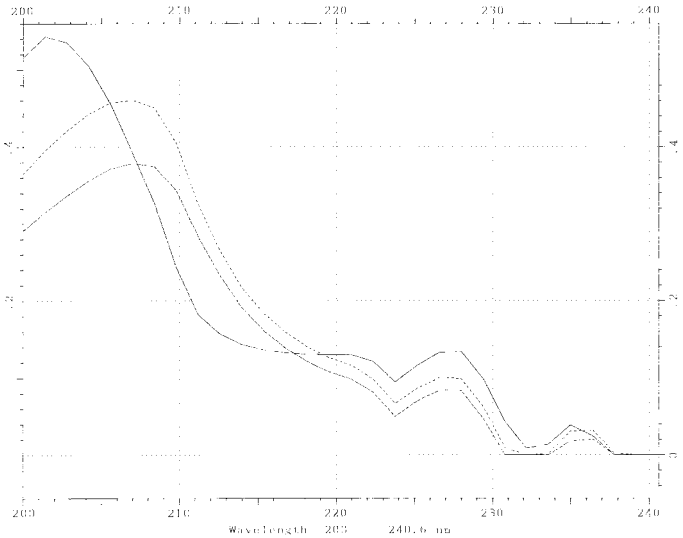

FIG. 2 UV Spectra of 2,4-D, 2,4,5-T, and Silvex Wavelength from 200 to 240 nm

processed as a safeguard against chronic laboratory contamination. The method blank must be carried through all stages of sample preparation and measurement.

10.8.3 The laboratory must, on an ongoing basis, analyze a replicate sample and a matrix spike or a matrix spike and a replicate matrix spike. This will allow the laboratory to assess method precision and accuracy.

11. Calculation

$$C_{samp} = \frac{H_{samp}}{H_{std}} \times C_{std} \times \frac{FV}{W} \quad (1)$$

where:

C_{samp} = concentration of compound in sample, µg/g,
C_{std} = concentration of compound in standard, µg/mL,
H_{samp} = peak height of compound in sample, *AU*,
H_{std} = peak height of compound in standard, *AU*, and
FV = final volume (mL).

NOTE 6—This includes the initial volume plus any subsequent dilutions. In this test method, as written for solids and organic liquids, the final volume (*FV*) would be 1000 mL, (100 mL for the initial volume multiplied by a 10 times dilution). This test method as written for aqueous samples would have a final volume of 100 mL.

W = weight of sample used, grams.

NOTE 7—For aqueous samples, convert millilitres to equal gram weight.

AU = absorbance units.

NOTE 8—Peak area may be substituted for peak height.

12. Precision and Bias

12.1 The data presented in Table 1 represents single-operator precision.

NOTE 9—All precision data were obtained using eight replicates.

13. Keywords

13.1 chlorophenoxy acid herbicides; 2,4-D; 2,4,5-T; chlorophenoxy acid herbicides in waste; HPLC; Silvex, 2,4,5-TP

TABLE 1 Precision and Bias

Compound	Concentration, ppm	Average Recovery, %	Single Operator Precision, % RSD
Matrix: Water/Oil Mix			
2,4-D	186	106	3.9
2,4,5-T	140	101	2.4
Silvex	195	115	1.7
Matrix: Soil			
2,4-D	199	102	3.3
2,4,5-T	185	113	2.4
Silvex	198	98	3.2
Matrix: PCB Oil			
2,4-D	199	100	5.0
2,4,5-T	185	118	2.8
Silvex	194	88	5.7
Matrix: Water Solvents			
2,4-D	953	105	1.8
2,4,5-T	943	103	2.9
Silvex	969	91	9.4