



# Standard Test Method for Chlorophenoxy Acid Herbicides in Waste Using HPLC<sup>1</sup>

This standard is issued under the fixed designation D5659; 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 reappraisal. A superscript epsilon ( $\epsilon$ ) indicates an editorial change since the last revision or reappraisal.

## 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, or cleanup, or combinations thereof.

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*:<sup>2</sup>  
[D1193 Specification for Reagent Water](#)

<sup>1</sup> This test method is under the jurisdiction of ASTM Committee D34 on Waste Management and is the direct responsibility of Subcommittee D34.02 on Discharge of Organic Wastes into Sanitary Sewers.

Current edition approved Nov. 1, 2014. Published December 2014. Originally approved in 1995. Last previous edition approved in 2006 as D5659 – 95(2006). DOI: 10.1520/D5659-14.

<sup>2</sup> For referenced ASTM standards, visit the ASTM website, [www.astm.org](http://www.astm.org), or contact ASTM Customer Service at [service@astm.org](mailto:service@astm.org). For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

## 2.2 EPA Documents:

[Method 8150 Chlorinated Herbicides, Test Methods for Evaluating Solid Waste Physical/Chemical Methods SW-846 Third Edition](#)<sup>3</sup>

[Method 8000A Gas Chromatography, Test Methods for Evaluating Solid Waste Physical/Chemical Methods, SW-846, 3rd Edition, Final Update 1](#)<sup>3</sup>

[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](#)<sup>3</sup>

## 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:

<sup>3</sup> Available from the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402.

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.

## 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- $\mu$ m particle size. Equivalent stainless steel or radial compression columns may be used.

6.4 *Guard Column*, C18, 4- $\mu$ m particle size.

6.5 *Injector*, manual injection valve, instrument auto-sampler, equipped with a 500- $\mu$ L 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- $\mu$ L 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- $\mu$ m 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.<sup>4</sup> Other grades may be used,

<sup>4</sup> *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.

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 D1193.

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- $\mu$ m 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. (**Warning**—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.