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Standard Test Methods for Phenolic Compounds in Water[1]

This standard is issued under the fixed designation D1783; 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.

This standard has been approved for use by agencies of the U.S. Department of Defense.

1. Scope

1.1 These test methods cover the preparation of the sample and the determination of the concentration of phenolic compounds in water. They are based on the color reaction of phenol (C_6H_5OH) with 4-aminoantipyrine and any color produced by the reaction of other phenolic compounds is reported as phenol. The concentration of phenol measured represents the minimum concentration of phenolic compounds present in the sample.

1.2 Phenolic compounds with a substituent in the para position may not quantitatively produce color with 4-aminoantipyrine. However, para substituents of phenol such as carboxyl, halogen, hydroxyl, methoxyl, or sulfonic acid groups do produce color with 4-aminoantipyrine.

1.3 These test methods address specific applications as follows:

	Range	Sections
Test Method A—Chloroform Extraction	0 to 100 µg/L	11 to 17
Test Method B—Direct Photometric	>0.1 mg/L (100 µg/L)	18 to 24

1.4 It is the users' responsibility to assure the validity of the standard test method for use in their particular matrix of interest.

1.5 *This standard does not purport to address all 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.* For specific hazard statements see 6.3.2 and 8.6.

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1129 Terminology Relating to Water

D1192 Guide for Equipment for Sampling Water and Steam in Closed Conduits (Withdrawn 2003)[3]

D1193 Specification for Reagent Water

D1293 Test Methods for pH of Water

D2777 Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water

D3370 Practices for Sampling Water from Closed Conduits

D5789 Practice for Writing Quality Control Specifications for Standard Test Methods for Organic Constituents (Withdrawn 2002)[3]

D5810 Guide for Spiking into Aqueous Samples

D5847 Practice for Writing Quality Control Specifications for Standard Test Methods for Water Analysis

3. Terminology

3.1 *Definitions*—For definitions of terms used in these test methods, refer to Terminology D1129.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *phenolic compounds*—hydroxy derivatives of benzene and its condensed nuclei.

4. Summary of Test Methods

4.1 Test Method A and Test Method B are photometric procedures based on the reaction of steam-distillable phenolic compounds with 4-aminoantipyrine.

4.2 Test Method A differs from Test Method B mainly in that the sample is extracted with chloroform, thereby providing 20-fold greater sensitivity.

4.3 Both procedures involve first separating the phenolic compounds from the background matrix by distillation. Due to the differing solubilities and boiling points of the various phenolic compounds, each phenolic comes over in the distillation at a different rate. Some phenolics will be substantially transferred near the beginning of the distillation and some will not start to distill until near the end. For this reason some phenolics may not have been quantitatively transferred to the receiving flask when the specified volume of distillate has been collected.

[1] These test methods are under the jurisdiction of D19 on Water and are the direct responsibility of Subcommittee D19.06 on Methods for Analysis for Organic Substances in Water.

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

[3] The last approved version of this historical standard is referenced on www.astm.org.

5. Significance and Use

5.1 Phenolic compounds are sometimes found in surface waters from natural and industrial sources. Their presence in streams and other waterways frequently will cause off flavor in fish tissue and other aquatic food.

5.2 Chlorination of waters containing phenols may produce chlorophenols that are odoriferous and objectionable tasting.

6. Interferences

6.1 Common interferences that may occur in waters are phenol-decomposing bacteria, reducing substances, and strongly alkaline conditions of the sample. Provisions incorporated in these test methods will minimize the effects of such interferences.

6.2 Treatment procedures required prior to the analysis for removal of interfering compounds may result in the unavoidable elimination or loss of certain types of phenolic compounds. It is beyond the scope of these test methods to describe procedures for overcoming all of the possible interferences that may be encountered in the test methods, particularly with highly contaminated water and industrial waste water. The procedures used must be revised to meet the specific requirements.

6.3 A few methods for eliminating certain interferences are suggested. (See Section 8 for descriptions of reagents required.)

6.3.1 *Oxidizing Agents*—If the sample smells of chlorine, or if iodine is liberated from potassium iodide on acidification of the sample, remove the oxidizing agents so indicated immediately after sampling. The presence of oxidizing agents in the sample may oxidize some or all of the phenols in a short time. Ferrous sulfate or sodium arsenite solution may be added to destroy all of the oxidizing substances. Excess ferrous sulfate or sodium arsenite do not interfere since they are removed in the distillation procedure.

6.3.2 *Sulfur Compounds*—Compounds that liberate hydrogen sulfide (H_2S) or sulfur dioxide (SO_2) on acidification may interfere with the phenol determination. Treatment of the acidified sample with copper sulfate usually eliminates such interferences. Acidify the sample with sulfuric acid (H_2SO_4) or hydrochloric acid (HCl) until just acid to methyl orange. Then add a sufficient quantity of copper sulfate ($CuSO_4$) solution to give a light blue color to the sample or until no more copper sulfide (CuS) precipitate is formed. Excessive amounts of H_2S or SO_2 may be removed from the acidified sample by a brief aeration treatment or stirring before the addition of the $CuSO_4$ solution or both. **Warning:** Acidification of certain samples may produce vigorous evolution of carbon dioxide (CO_2), SO_2, H_2S, or other gases. Therefore, perform the acidification cautiously and stir the samples during the process. Complete the evolution of gases before the sample is stoppered.

6.3.3 *Oils and Tars*—If the sample contains oil or tar, some phenolic compounds may be dissolved in these materials. An alkaline extraction, in the absence of $CuSO_4$, may be used to eliminate the tar and oil. Adjust the pH of the sample between 12 and 12.5 with sodium hydroxide (NaOH) pellets to avoid extraction of the phenols. Extract the mixture with carbon tetrachloride (CCl_4). Discard the oil- or tar-containing layer. Remove any CCl_4 remaining in the aqueous portion of the sample by gentle heating.

NOTE 1—The presence of $CuSO_4$ is detrimental since it is converted to cupric hydroxide ($Cu(OH)_2$) by the NaOH. The $Cu(OH)_2$ acts as an oxidizing agent on phenols.

7. Apparatus

7.1 *Buchner-Type Funnel with Coarse Fritted Disk*—At least three funnels are needed for determination of phenolic compounds by Test Method A. Alternatively, standard glass funnels and pre-fluted filter paper may be used. The funnel paper must be large enough to hold 5 g of sodium sulfate. These funnels are not used in Test Method B.

7.2 *Photometer*—A spectrophotometer or filter photometer, suitable for use at 460 nm (Test Method A) or at 510 nm (Test Method B), and accommodating a cell that gives a light path of 1.0 to 10 cm shall be used. The size of the cell used will depend on the absorbance of the colored solutions being measured and the characteristics of the photometer. In general, if the absorbances are greater than 1.0 with a larger cell, the next smaller size cell should be used.

7.3 *Distillation Apparatus*—A 1-L, heat-resistant, distilling flask attached to a Graham condenser by means of a glass joint.

7.4 *pH Meter*—This apparatus shall conform to the requirements in Test Methods D1293.

8. Reagents

8.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.[4] 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.

8.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean water conforming to Specification D1193 Types I, II, III, or IV. Water used for these test methods shall be free of phenolic compounds, residual chlorine, and substances that interfere with the test. Water sufficiently free of phenolics can be generated by boiling the water for 20 min.

8.3 *Aminoantipyrine Solution* (20 g/L)—Dissolve 2.0 g of 4-aminoantipyrine in water and dilute to 100 mL. Prepare this reagent fresh as used.

NOTE 2—The melting point of a satisfactory grade of 4-aminoantipyrine ranges from 108.0 to 109.5°C.

8.4 *Ammonium Chloride Solution* (20 g/L)—Dissolve 20 g of ammonium chloride (NH_4Cl) in water and dilute to 1 L.

[4] *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.

8.5 *Ammonium Hydroxide* (NH_4OH) (sp gr 0.90)—Concentrated ammonium hydroxide (NH_4OH).

8.6 *Carbon Tetrachloride* (CCl_4). **Warning:** Phenol, carbon tetrachloride, and chloroform are potentially hazardous to human health. Avoid inhalation and direct contact. Use in a well-ventilated hood.

8.7 *Chloroform* ($CHCl_3$).

8.8 *Hydrochloric Acid* (HCl) (sp gr 1.19)—Concentrated hydrochloric acid (HCl).

8.9 *Phenol Solution, Stock* (1 mL = 10 mg phenol)—Dissolve 1.00 g of phenol (C_6H_5OH) in freshly boiled and cooled water. Dilute to 1 000 mL with freshly boiled cooled water. Prepare a fresh stock solution within 30 days of use.

8.10 *Phenol Solution, Intermediate (C_6H_5OH) (1 mL = 10 µg phenol)*—Dilute 10.0 mL of the stock solution to 1 000 mL with freshly boiled and cooled water. Prepare this solution fresh on the day it is used.

8.11 *Phenol Solution, Standard (C_6H_5OH) (1 mL = 1.0 µg phenol)*—Dilute 50 mL of the intermediate solution to 500 mL with freshly boiled and cooled water. Prepare this solution fresh within 2 h of use.

8.12 *Potassium Ferricyanide Solution*($K_3Fe(CN)_6$) (80 g/L)—Dissolve 8.0 g of ($K_3Fe(CN)_6$) in water and dilute to 100 mL. Filter if necessary. Prepare fresh weekly.

8.13 *Sodium Bisulfate* ($NaHSO_4$).

8.14 *Sodium Sulfate* (Na_2SO_4), anhydrous and granular.

8.15 *Sulfuric Acid* (H_2SO_4) (sp gr 1.84)—Concentrated sulfuric acid (H_2SO_4).

8.16 *Sulfuric Acid Solution* (H_2SO_4) (1+9)—Cautiously add one volume of concentrated H_2SO_4 to nine volumes of water with continuous cooling and mixing. Solution will become hot.

9. Sampling

9.1 Collect the sample in accordance with Specification D1192 and Practices D3370.

9.2 When samples are composited, chill the samples or the composite sample immediately and keep at a temperature of not more than 4°C during the compositing period. The collection time for a single composite sample shall not exceed 4 h. If longer sampling periods are necessary, collect a series of composite samples. Then preserve such composite samples in accordance with Section 10 until analyzed.

10. Preservation of Samples

10.1 Phenolic compounds in water are subject to both chemical and biochemical oxidation. Preserve samples within 4 h of collection. Acidify the samples to a pH between 0.5 and 2.0 with H_3PO_4, HCl, H_2SO_4, or $NaHSO_4$.

10.2 To further minimize any changes in the phenolic content of the sample, keep it cold, preferably between 2°C and 4°C until analysis. The preserved samples should be in glass, not plastic bottles, and preferably analyzed within 28 days after collection.

TEST METHOD A—CHLOROFORM EXTRACTION

11. Scope

11.1 This test method is generally applicable to water that contains less than 100 µg/L (0.1 mg/L) of phenolic compounds. Lower levels may be achieved with different instruments and larger cells. Higher levels can be achieved by dilution.

11.2 The lowest levels of analyte detection or accurate quantitation are laboratory and sample matrix dependent and it is up to the users of the test method to determine these levels in their own situation.

11.3 This test method was tested on municipal wastewater treatment plant influent and effluent, lake water, river water, and industrial treatment plant effluent. It is the user's responsibility to insure the validity of this test method for waters of untested matrices.

12. Summary of Test Method

12.1 This is a photometric test method, based on the reaction of steam-distillable phenolic compounds with 4-aminoantipyrine at a pH of 10.0 ± 0.2 in the presence of $K_3Fe(CN)_6$. The antipyrine dye formed is extracted from the aqueous solution with chloroform and the absorbance is measured at 460 nm. The concentration of phenolic compounds in the sample is expressed in terms of micrograms per litre of phenol C_6H_5OH.

13. Calibration

13.1 Prepare a series of 500-mL C_6H_5OH standards in freshly boiled and cooled water containing 0, 5, 10, 20, 30, 40, and 50 mL of standard C_6H_5OH solution (1 mL = 1.0 µg C_6H_5OH). Use all solutions at room temperature.

13.2 Develop color in the series of standards and prepare the chloroform extracts in accordance with the procedures prescribed in Section 14 and 15.

13.3 Measure the absorbance of each standard at 460 nm against the reagent method blank (blank) as zero absorbance. Plot the absorbances against the corresponding weights in micrograms of phenol.

NOTE 3—Make a separate calibration curve for each spectrophotometer or photoelectric colorimeter. Check each curve periodically to ensure reproducibility.

14. Distillation Procedure

14.1 Measure 500 mL of the sample into a beaker. Adjust the pH of the sample to between pH 0.5 and 4 with H_2SO_4 solution (1+9). Use methyl orange indicator solution or a pH meter to aid in the pH adjustment. If the sample has been previously preserved according to 10.1, this pH adjustment step may be omitted. Transfer the mixture to the distillation apparatus. Use a 500-mL graduated cylinder as a receiver.

14.2 Distill 450 mL of the sample. Stop the distillation and, when boiling ceases, add 50 mL of water to the distillation flask. Continue the distillation until a total of 500 mL has been collected.

14.3 If the distillate is turbid, a second distillation may prove helpful. Acidify the turbid distillate with H_2SO_4 solution

TABLE 1 Precision Data—Test Method A

Level	Reagent Water Matrix			Optional Water Matrix		
	6.460 µg/L	34.780 µg/L	67.900 µg/L	5.430 µg/L	32.840 µg/L	66.260 µg/L
n	23	23	23	24	24	23
S_T	3.384	4.190	8.923	2.494	3.957	8.147
S_o	2.718	5.320	7.300	2.528	3.243	5.850
So:C.V. [A]	38 %	10.8 %	11.8 %	46.6 %	9.9 %	8.8 %

[A] Coefficient of variation (S_o level) by 100.

(1+9) and repeat the previously described distillation. The second distillation usually eliminates the turbidity. However, if the second distillate is also turbid, the screening procedure must be modified. Attempt an extraction process before the distillation to avoid turbidity in the distillate.

15. Determination of Phenolic Compounds

15.1 Transfer to a beaker the 500 mL of distillate, or a suitable aliquot diluted to 500 mL containing no more than 50 µg of phenolic compounds. The distillate and all solutions used must be at room temperature. Trial and error tests may be necessary to determine the volume of a suitable aliquot. Also, prepare a blank consisting of 500 mL of water.

15.2 Add 25 mL of NH_4Cl solution to each aliquot. Adjust the pH between 9.8 and 10.2 with NH_4OH. Transfer each mixture to a 1-L separatory funnel. Add 3.0 mL of 4-aminoantipyrine solution (20 g/L) and mix immediately, then add 3.0 mL of $K_3Fe(CN)_6$ solution and again mix immediately. Allow color to develop for 3 min.

NOTE 4—The solutions should be clear and have a light yellow color. If not, an interfering substance is indicated. Repeat the determination after more complete treatment to eliminate the interference.

15.3 Pipet 25.0 mL of chloroform into each separatory funnel if a 1.0 to 5.0-cm cell is to be used in the colorimeter. Add 50.0 mL if a 10-cm cell is to be used. Shake the separatory funnel ten times. When the chloroform has settled, again shake the separatory funnel ten times and allow the chloroform to settle.

15.4 Filter each of the chloroform extracts through separate fritted-glass funnels or fluted filter paper in standard funnels containing 5 g of anhydrous, granular Na_2SO_4 directly into clean absorption cells as needed for absorbance measurements. Do not add additional chloroform.

15.5 Using the chloroform extract of the reagent blank adjust the colorimeter to zero absorbance at 460 nm. Measure the absorbance of the sample extract at the same wavelength. By reference to the calibration curve (Section 13) and the absorbance obtained on the sample extract, determine the phenolic content of the sample.

16. Calculation

16.1 Calculate the phenolic content of the sample, in micrograms per litre, as follows:

$$\text{Concentration of phenolics in original sample } \mu g/L = W \times 100/V$$

where:

W = phenolics, in aliquot of sample distillate diluted to 500 mL as determined from calibration curve, µg, and

V = sample distillate, in the 500-mL solution reacted with 4-aminoantipyrine, mL.

NOTE 5—Since the ratio of the various phenolic compounds present in a given sample is unpredictable, phenol (C_6H_5OH) is used as a standard. Any color produced by the reaction of other phenolic compounds is reported as phenol. This value will represent the minimum concentration of phenolic compounds present in the sample.

17. Precision and Bias[5]

17.1 Eight laboratories participated in a collaborative study to determine the precision and bias of this procedure. The study was conducted by sending C_6H_5OH concentrates to participating laboratories. The laboratories then spiked these concentrates into phenol free reagent grade water and an optional water matrix of their choice. The precision and bias values determined in this study include any variability due to make up, splitting, shipment, and dilution of the concentrates used.

17.2 The optional water matrices chosen by the participants included: river water (2), municipal wastewater treatment plant effluent (3), lake water (1), raw sewage (1), and industrial wastewater treatment plant effluent (1). All of the data from the optional matrix portion of the study was combined to obtain composite precision values. None of the matrices used seemed to have a greater effect on precision than any other, but they did have the effect of degrading recovery (bias). The precision on samples in the optional matrix was comparable to that obtained with the reagent water matrix.

17.3 The collaborative study and data analysis was performed using Practice D2777. Within each matrix, each laboratory analyzed three concentration levels, each in triplicate.

17.4 The final precision data are summarized in Table 1,

where:

S_T = between laboratory standard deviation, and

S_O = within laboratory standard deviation from geometric mean of weighted individual laboratory variances).

The precision of this test method depends in part on the interferences present and the skill of the analyst.

17.5 The bias of the test method, as indicated from the collaborative study, is summarized in Table 2. This data is

[5] Supporting data have been filed at ASTM International Headquarters and may be obtained by requesting Research Report RR:D19-1132.