Designation: ~~D6363 – 98 (Reapproved 2013)~~ D6363 – 20

Standard Test Method for Determination of Hydrogen Peroxide and Combined Organic Peroxides in Atmospheric Water Samples by Peroxidase Enzyme Fluorescence Method[1]

This standard is issued under the fixed designation D6363; 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 determination of hydroperoxides, which include hydrogen peroxide (H_2O_2) and combined organic peroxides, in samples of atmospheric water by the method of horseradish peroxidase derivatization and fluorescence analysis of the derived dimer.[2,3]

1.2 The range of applicable hydrogen peroxide concentrations was determined to be 0.6–176.0 × 10^{-6} M from independent laboratory tests of the test method.

1.3 The primary use of the test method is for hydrogen peroxide, but it may also be used to quantitate organic hydroperoxides. Determinations of organic hydroperoxide concentration levels up to 30 × 10^{-6} M may be adequately obtained by calibration with hydrogen peroxide.[2,3] While organic hydroperoxides have not been detected at significant concentration levels in rain or cloud water, their presence may be tested by operation of the test method with the addition of catalase for destruction of H_2O_2.[3]

1.4 Because of the instability of hydroperoxides in atmospheric water samples, proper sample collection, at-collection derivatization, and stringent quality control are essential aspects of the analytical process.

1.5 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.6 *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~~ safety, health, and ~~health~~environmental practices and determine the applicability of regulatory limitations prior to use.*

1.7 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

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[1] This ~~guide~~ test method is under the jurisdiction of ASTM Committee D22 on Air Quality and is the direct responsibility of Subcommittee D22.03 on Ambient Atmospheres and Source Emissions.

Current edition approved ~~Oct. 1, 2013~~Sept. 1, 2020. Published ~~October 2013~~September 2020. Originally approved in 1998. Last previous edition approved in ~~2009~~2013 as D6363 – 98 ~~(2009)~~(2013).~~ε1~~. DOI: ~~10.1520/D6363-98R13.~~10.1520/D6363-20.

[2] ~~Lazrus, A. L., Kok, G. L., Gitlin, S. N., and Lind, J. A., "Automated Fluorometric Method for Hydrogen Peroxide in Atmospheric Precipitation," *Anal. Chem.*, 57, 1985, pp. 917–922.~~Lazrus, A. L., Kok, G. L., Gitlin, S. N., and Lind, J. A., "Automated Fluorometric Method for Hydrogen Peroxide in Atmospheric Precipitation," *Analytical Chemistry*, Vol 57, 1985, pp. 917–922.

[3] ~~Kok, G. L., Thompson, K., and Lazrus, A. L., "Derivatization Technique for the Determination of Peroxides in Precipitation," *Anal. Chem.*, 58, 1986, pp. 1192–1194.~~Kok, G. L., Thompson, K., and Lazrus, A. L., "Derivatization Technique for the Determination of Peroxides in Precipitation," *Analytical Chemistry*, Vol 58, 1986, pp. 1192–1194.

2. Referenced Documents

2.1 *ASTM Standards:*[4]

D1129 Terminology Relating to Water
D1193 Specification for Reagent Water
D1356 Terminology Relating to Sampling and Analysis of Atmospheres
D5012 Guide for Preparation of Materials Used for the Collection and Preservation of Atmospheric Wet Deposition
D5111 Guide for Choosing Locations and Sampling Methods to Monitor Atmospheric Deposition at Non-Urban Locations
E200 Practice for Preparation, Standardization, and Storage of Standard and Reagent Solutions for Chemical Analysis

3. Terminology

3.1 *Definitions*—For definitions of terms used in this test method, refer to Terminologies D1129 and D1356 and Guide D5111.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *atmospheric water, n*—liquid or solid water suspended in the atmosphere or deposited from the atmosphere. Forms of atmospheric water include rain, snow, fog, cloud water, dew, and frost.

3.2.2 *derivatization, n*—formation of the p-hydroxyphenylacetic acidic dimer by combination of p-hydroxyphenylacetic acid, horseradish peroxidase reagent, and hydroperoxide(s). Also the procedure of addition of the derivatizing reagent to samples.

3.2.3 *hydroperoxides, n*—hydrogen peroxide and organic peroxides dissolved in water.

3.2.4 *intrinsic hydroperoxides, n*—hydroperoxides contained in reagent water used for the method.

3.2.5 *post-derivatization, n*—addition of the derivatizing reagent to the sample after collection.

3.2.6 *pre-derivatization, n*—addition of the derivatizing reagent to the sample collection container prior to sample collection.

3.2.7 *systems blank, n*—a field blank of reagent water that is subjected to a similar or identical environment and derivatization time as a collected atmospheric water sample.

3.2.8 *systems standard, n*—a H_2O_2 calibration standard solution subjected to a similar or identical environment and derivatization time as a collected atmospheric water sample.

4. Summary of Test Method

4.1 The peroxidase enzyme fluorescence method is based on the reaction of hydroperoxides, horseradish peroxidase, and p-hydroxyphenylacetic (PHOPAA) acid, forming a fluorescent dimer of the latter. This dimer is detected using a fluorometric technique, and the hydroperoxides are quantified by calibration with hydrogen peroxide. The formation of the dimer (derivatization) shall be accomplished soon after sample collection to minimize H_2O_2 decay. In addition, strict quality assurance practices are part of the method, including use of systems standards and systems blanks to estimate hydroperoxide loss and to assess derivatizing solution effectiveness.

5. Significance and Use

5.1 Hydrogen peroxide (formed photochemically in the atmosphere) is a primary oxidizer of dissolved sulfur dioxide in atmospheric water. Detection of H_2O_2 in atmospheric water is useful for inferring gas-phase H_2O_2 concentrations and for assessing the relative importance of various acidifying mechanisms under specific atmospheric conditions.

5.2 Hydroperoxides in samples to be analyzed are unstable in water and can decay rapidly due to bacterial action or chemical reaction with other constituents. The test method includes procedures for sample derivatization and methods for estimating and correcting for hydroperoxide decay.

[4] 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.

6. Interferences

6.1 The derivatizing reagent is formulated to counteract the effects of the following potentially interfering species.

6.2 *Hydroxymethane Sulfonate (HMSA)*—The addition of formaldehyde (HCHO) to the derivatizing reagent will suppress the negative interference of HMSA. In the absence of added HCHO, the PHOPAA dimer in a derivatized simulated rain sample, containing 1.2×10^{-5}M H_2O_2 and 1.0×10^{-4}M HMSA, displayed a fluorescence signal 5 % lower than that observed when HCHO was added to the derivatizing reagent.[3]

6.3 *Trace Transition Metals and Common Ionic Components of Atmospheric Water (Sodium, Ammonium, Hydrogen, Sulfate, Nitrate, Chloride, Formate)*—Potential interference by transition metals is overcome by the formation of ethylenediaminetetraacetic acid (EDTA) complexes. Tests of simulated rain samples containing transition metals and common ionic components of precipitation have demonstrated both the general applicability of this test method to samples containing common contaminants and the stability of derivatized solutions stored at 4°C for more than five days.[3]

7. Apparatus

7.1 *Flow System,* consisting of the following:

7.1.1 *Automatic sampler or injection valve.*

7.1.2 *Automated wet chemistry (peristaltic) pump.*

7.1.3 *Reagent manifold.*

7.1.4 *Mixing coil,* 5-turn, 2-mm inner diameter.

7.1.5 *Fluorometer,* excitation at 320 nm and measurement of the fluorescence signal at 400 nm, flow-through fluorescence cell.

7.1.6 *Recorder.*

7.2 *Sample and Standards Containers*—All containers used for sample collection and sample transport, for storage and analysis of samples and standards, and for reagents should be high density polyethylene, TFE-fluorocarbon, or borosilicate glass, cleaned in accordance with procedures established for analyses of common inorganic ions (see Guide D5012).

7.3 *Pipettes with Disposable Tips*—Solution preparation and sample fixing operations are generally conducted using automatic pipettes. Solution volumes delivered by these devices should be verified to confirm consistent and accurate performance.[5]

7.4 *Reagent Bottles*—All containers used for the preparation and storage of derivatizing and other reagent solutions shall be dedicated for hydroperoxides. Containers for solutions of catalase shall not be used for non-catalase solutions.

8. Reagents and Materials

8.1 *Purity of Reagents*—Unless otherwise noted, reagent grade chemicals shall be used.[6]

8.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water as defined by Type I of Specification D1193, with the added stipulation that the total organic carbon content be less than 20 µg/L. A Type I water system equipped with an organic extraction cartridge and a 0.2 µm filter is an acceptable water source. Water to be used for reagents, standard solutions, and analytical rinsing should be stored in borosilicate glass.

[5] ~~Schwartz, L.M., "Calibration of Pipets: A Statistical View," *Analytical Chemistry*, Vol. 61, 1989, pp. 1080–1083.~~ Schwartz, L.M., "Calibration of Pipets: A Statistical View," *Analytical Chemistry*, Vol 61, 1989, pp. 1080–1083.

[6] ~~*Reagent Chemicals, American Chemical Society Specifications,*~~*ACS Reagent Chemicals, Specifications and Procedures for Reagents and Standard-Grade Reference Materials,* 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. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

8.3 *Catalase Enzyme* (1.7×10^6 units/mL)[7]—The enzyme catalase may be used for the destruction of H_2O_2 in atmospheric water samples. Its addition to the sample before addition of the derivatizing reagent removes H_2O_2, but organic hydroperoxides are preserved. Subsequent addition of the derivatizing reagent results in dimer formation by way of reaction with peroxides other than H_2O_2. Results of analyses of catalase-treated samples may be compared with the measurement of peroxides in samples without catalase to determine H_2O_2 by difference.

8.3.1 *Catalase,* 1 + 49—Dilute 1 mL of catalase enzyme to a final volume of 50 mL with water. Before pipetting the concentrated solution, ensure that all the solid material is completely suspended by shaking or stirring the bottle of concentrate. Allow the dilute solution to stand at least 4 h before use. The solution can be stored for up to 48 h at 4°C.

8.4 *Derivatizing Reagent, Concentrated*—Dissolve 12.11 g of Tris(hydroxymethyl)aminomethane, 0.38 g of EDTA, tetrasodium salt, 4.57 g of PHOPAA, 300 units of horseradish peroxidase, and 1 mL concentrated hydrochloric acid in water, and dilute to 200 mL in a volumetric flask. The final pH of this solution should be 9.0. If greater than 9.5 or less than 8.5, remake. Prepare every four days and store at 4°C. Measurement of peroxides in aqueous atmospheric samples is based on the fluorescence of the PHOPAA dimer produced by reaction of hydroperoxides with PHOPAA. The fluorescence of samples derivatized at the time of collection provides a measure of total hydroperoxide (organic and H_2O_2) content of the sample.

8.4.1 *Derivatizing Reagent, 4 + 96*—Dilute 4.0 mL of the concentrated derivatizing reagent to 100 mL with water. Prepare daily as needed, and keep tightly sealed at 4°C.

NOTE 1—The dilute derivatizing reagent is normally added to samples to be analyzed in the reagent:sample ratio of 1:1. Other concentrations of dilute derivatizing reagent may be used as long as the final ratio entering the analytical system is 1:1. Under special circumstances, other ratios may be dictated by sampling conditions (see 10.6 and 10.7).

8.5 *Hydrochloric Acid* (HCl), (1 M)—Add 8.3 mL concentrated HCl to water in a volumetric flask and dilute to 100 mL.

8.6 *Peroxide Solution, Standard Stock* (1 %)—Dilute commercially available (pharmaceutical grade is acceptable) H_2O_2 solution (30 %) approximately 1 + 29 with water in a volumetric flask. Add sodium stannate (Na_2SnO_3) to a concentration of 10.65 mg/L and store at 4°C, and store in a borosilicate glass bottle. Determine the peroxide concentration by titration with standard permanganate solution (see 11.2) approximately 24 h after preparation. Update the concentration determination by titration at one month intervals.

8.6.1 *Peroxide Solution,* 1 + 199—Dilute 500 µL of the standard stock (1 %) solution to 100 mL with water in a volumetric flask. The approximate H_2O_2 concentration of the resulting solution is 1500 µM (50 mg/L). Calibration standards are prepared immediately before sample analysis by diluting aliquots of this solution (see 11.3).

8.6.2 *Peroxide Solution, Systems Blank*—Water combined with dilute derivatizing reagent to the ratio 1:1. Prepare in peroxide calibration standard vials or in sample collection containers, depending on the derivatization method (see Section 10).

8.6.3 *Peroxide Solution, Systems Standard*—See 11.3. Prepare in vials used for peroxide calibration standards or in sample collection containers, depending on the derivatization method (see Section 10).

8.7 *Potassium Permanganate* ($KMnO_4$)*Solution, Standard* (0.01 M)—Dissolve 1.58 g $KMnO_4$ in 100 mL water, and dilute the solution with water to 1 L. Seal tightly, and store in an amber borosilicate glass bottle in the dark. Standardize following the procedure in Practice E200, Sections 64–68; adjust chemical proportions according to 9.1 of that Practice.

8.8 *Sodium Hydroxide* (NaOH) (0.1 M)—Dissolve 4.0 g of sodium hydroxide in water and dilute to 1 L. Prepare weekly.

8.9 *Sulfuric Acid* (H_2SO_4), 5 % (3.6 M)—Add 5 mL concentrated H_2SO_4 to water in a volumetric flask, and dilute to 100 mL.

[7] Catalase enzyme, 1.7×10^6 units/mL, has been found satisfactory for this purpose. Available through Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178.

9. Sample Collection

9.1 Select sampling locations and sampling methods in accordance with Guide D5111. Additional considerations specific to sampling for aqueous-phase hydrogen peroxide are provided in 9.3 and 9.4.

9.2 Methods of preparation of sample containers for collection, transport, and storage shall be those detailed in Guide D5012 under inorganic ionic species (see 8.1 and 8.2 of Guide D5012).

9.3 Control procedures designed to ensure sample integrity in the field (see Section 10) are difficult to perform adequately if buckets or other high atmospheric-exposure collectors are used. Therefore, sampling for rain should be conducted using funnel-and-bottle type, or narrow-necked, collectors.

9.4 The requirements for controlled derivatization of hydroperoxides and timely analysis (see Section 10) dictate that sampling for wet deposition be conducted on a daily or more frequent basis.

10. Derivatization

10.1 The following procedures shall be in addition to those specified for preservation of inorganic anions and cations in Guide D5012 (see Table 1 of Guide D5012).

10.2 Hydroperoxides dissolved in atmospheric water solutions are subject to decay at rates that are not predictable. Therefore, the derivatizing solution shall be added during sample collection or within a known and controlled time after sample collection.

10.3 The rate of decay of non-derivatized hydroperoxides may be quite fast: loss rates ranging from 1 to 28 % h^{-1} were found for rain samples collected at Boulder, CO.[2] The decay may be significant during the time of sampling, an especially important consideration for sampling of precipitation. Thus, addition of the derivatizing reagent to the collection container prior to sampling (pre-derivatization) is the most desirable method. The pre-derivatized sample, however, is not suitable for analysis for other species, particularly hydrogen ion, ammonium, and alkali metals.

10.4 Derivatization of samples following sample collection (post-derivatization) is an acceptable method and is required if the collected sample must be used for analyses for other species. Standardization procedures additional to those used for pre-derivatization shall be applied. Samples from different locations whose hydroperoxide concentrations are to be compared shall be treated as close to identically as possible.

10.5 Derivatized solutions (samples, systems standards, and systems blanks) should be labeled, sealed, and stored on ice for transport to the analytical laboratory. The efficacy of the derivatizing reagent decreases with time in all types of solutions: reagents, samples, standards, and blanks. Systems standards and blanks are prepared and exposed to sampling conditions as nearly identical to those experienced by the samples as possible. Tests with simulated precipitation samples indicate that derivatized samples may be stored at 4°C for up to five days before analysis.[2] These results may not be generally applicable to actual atmospheric water samples, however. Consequently, storage at 4°C and analysis within 24 h are strongly recommended to reduce the potential for significant sample deterioration. Reanalysis of samples over a period of several days is suggested to establish the sample deterioration pattern for samples from varied environments.

10.6 *Pre-Derivatization Procedure:*

10.6.1 The dilute derivatizing reagent concentration is determined so that its addition to the sample results in a 1:1 dilution of the sample. The derivatized sample is then suitable for direct injection into the analytical stream. To arrive at this final solution, the volume of sample collected shall be appropriately controlled.

10.6.2 When the sample volume cannot be controlled, an estimate of expected sample volume should be made to correspond with the amount of derivatizing reagent added.

10.6.3 When the required volume of dilute reagent would be too large (for example, if the volume of resulting sample might overflow the collection container), use of an intermediate concentration of derivatizing reagent between that of the concentrated and the dilute is acceptable as long as the final dilution is expected to be 1:1.