Designation: D6363 – 98 (Reapproved 2013)

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 *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.*

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.

[1] This guide 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.

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[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.

[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.

[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.

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.

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.

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.

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

[6] *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. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

[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.