



# Standard Test Method for Determination of Hydrogen Peroxide and Combined Organic Peroxides in Atmospheric Water Samples by Peroxidase Enzyme Fluorescence Method<sup>1</sup>

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## 1. Scope

1.1 This test method covers the determination of hydroperoxides, which include hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and combined organic peroxides, in samples of atmospheric water by the method of horseradish peroxidase derivatization and fluorescence analysis of the derived dimer.<sup>2,3</sup>

1.2 The range of applicable hydrogen peroxide concentrations was determined to be  $0.6 - 176.0 \times 10^{-6}$  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  $30 \times 10^{-6}$  M may be adequately obtained by calibration with hydrogen peroxide.<sup>2,3</sup> 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  $\text{H}_2\text{O}_2$ .<sup>3</sup>

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.*

<sup>1</sup> 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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<sup>2</sup> 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.

<sup>3</sup> 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.

## 2. Referenced Documents

- 2.1 *ASTM Standards*:<sup>4</sup>
- 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.

<sup>4</sup> 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  $\text{H}_2\text{O}_2$  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  $\text{H}_2\text{O}_2$  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  $\text{H}_2\text{O}_2$  in atmospheric water is useful for inferring gas-phase  $\text{H}_2\text{O}_2$  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  $1.2 \times 10^{-5}\text{M}$   $\text{H}_2\text{O}_2$  and  $1.0 \times 10^{-4}\text{M}$  HMSA, displayed a fluorescence signal 5 % lower than that observed when HCHO was added to the derivatizing reagent.<sup>3</sup>

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.<sup>3</sup>

#### 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.<sup>5</sup>

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.<sup>6</sup>

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  $\mu\text{g/L}$ . A Type I water system equipped with an organic extraction cartridge and a 0.2  $\mu\text{m}$  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* ( $1.7 \times 10^6$  units/mL)<sup>7</sup>—The enzyme catalase may be used for the destruction of  $\text{H}_2\text{O}_2$  in atmospheric water samples. Its addition to the sample before addition of the derivatizing reagent removes  $\text{H}_2\text{O}_2$ , but organic hydroperoxides are preserved. Subsequent addition of the derivatizing reagent results in dimer formation by way of reaction with peroxides other than  $\text{H}_2\text{O}_2$ . Results of analyses of catalase-treated samples may be compared with the measurement of peroxides in samples without catalase to determine  $\text{H}_2\text{O}_2$  by difference.

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

<sup>6</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. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

<sup>7</sup> Catalase enzyme,  $1.7 \times 10^6$  units/mL, has been found satisfactory for this purpose. Available through Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178.

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<sub>2</sub>O<sub>2</sub>) 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<sub>2</sub>O<sub>2</sub> solution (30 %) approximately 1 + 29 with water in a volumetric flask. Add sodium stannate (Na<sub>2</sub>SnO<sub>3</sub>) 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<sub>2</sub>O<sub>2</sub> 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<sub>4</sub>) Solution, Standard (0.01 M)*—Dissolve 1.58 g KMnO<sub>4</sub> 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<sub>2</sub>SO<sub>4</sub>), 5 % (3.6 M)*—Add 5 mL concentrated H<sub>2</sub>SO<sub>4</sub> to water in a volumetric flask, and dilute to 100 mL.

## 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<sup>-1</sup> were found for rain samples collected at Boulder, CO.<sup>2</sup> 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.<sup>2</sup> 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.

NOTE 2—In the case of automated collection of rain samples, the volume may be controlled by use of a fixed-volume sequential sampler.

10.6.4 Samples whose final volumes are less than that desired (reagent:sample > 1:1) should be diluted to the 1:1 level with water (from the same batch as that used for reagents, standards, and analytical solutions) prior to analysis. The analytical results should be adjusted to reflect the additional dilution.

10.6.5 If the sample volume exceeds the expected (reagent:sample < 1:1) by more than 10 %, derivatization of the sample might be less than complete, or the effective baseline for the analytical procedure may be changed. Therefore, calibration standards and analytical blanks should be prepared with approximately the same ratio and analyzed along with the samples and the normal calibration standards and blanks. Determined sample concentrations may be adjusted to correspond to any differences in results between using the 1:1 standards and using those approximating the ratio of the samples.

10.6.6 Two systems blanks, and at least three systems standards, with concentrations suggested in Section 11 or others bracketing the hydroperoxide levels anticipated, shall be prepared and placed in containers identical to the sample collection containers at the same time as the addition of derivatizing reagent to the sample collection containers. The volumes of the systems standards and blanks should approximate that of the expected collected samples. These containers should be sealed and handled exactly as sample collection

containers except for the introduction of a sample. These standards and blanks are analyzed as additional samples, providing an estimate of sampling environment effects on reagent efficacy. Corrections may be made to actual sample concentrations as in 10.6.5.

#### 10.7 Post-derivatization Procedure:

10.7.1 Derivatization of samples shall be conducted at the completion of sample collection, at the sampling site, to minimize post-sampling decay of hydroperoxides.

10.7.2 Derivatized samples are prepared by combining equal volumes of the collected sample and the dilute derivatizing reagent in high-density polyethylene or borosilicate glass vials of appropriate size. Use of disposable pipette tips is strongly recommended, especially if the remainder of the collected sample is to be analyzed for other species. The minimum volume required for the analytical procedure described in Section 12 is 4 mL (2 mL reagent plus 2 mL sample). Smaller volumes are diluted to the minimum with systems blank solution, and analytical results are adjusted to reflect the additional dilution.

10.7.3 At least three systems standard solutions and two systems blanks should be prepared as soon as possible before derivatizing samples. The standard preparation is from the stock standard to minimize peroxide decay in the standard during the time between preparation of the systems standards and derivatization of the samples. The systems standards and blanks use vials from the same batch as those used for samples. Results of analyses of the systems standards will be used to estimate post-derivatization, pre-analysis decay and to allow adjustment to sample results as in 10.6.5.

## 11. Calibration and Standardization

11.1 Perform calibration of the analysis system using dilute H<sub>2</sub>O<sub>2</sub> solutions prepared by serial dilution of the 1:200 standard. Prepare these solutions during the same day as analysis of the samples. Label and seal the standards, and store at 4°C with the atmospheric water samples until analysis. Measure the fluorescences of the standards before introduction of the samples; check selected standards periodically (after the analysis of every 10 to 15 samples) to demonstrate system stability. Choose standard concentrations to span the expected concentration range of the atmospheric water samples.

11.2 *Standardization of the Stock H<sub>2</sub>O<sub>2</sub> Solution*—Dilute 1.00 mL of the stock peroxide solution to 50 mL of 5 % H<sub>2</sub>SO<sub>4</sub> in a volumetric flask. Titrate with standard KMnO<sub>4</sub> (0.01 M). Although the solution may take on a dark pink color after the initial permanganate addition, it will become colorless after a few seconds and will remain colorless until the end point, signaled by a persistent pink color, is reached. Add permanganate drop-wise as the equivalence point is approached to avoid overshooting the end point. Calculate the concentration of the peroxide solution as follows:

$$H = 2.5 AB/C \quad (1)$$

where:

$H$  = H<sub>2</sub>O<sub>2</sub> concentration, M,  
 $A$  = volume KMnO<sub>4</sub> added to reach end point, mL,  
 $B$  = concentration of KMnO<sub>4</sub>, M, and

$C$  = volume of standard  $H_2O_2$ , mL.

Perform two additional standardizations, and calculate the peroxide concentration from the average of the three titrations.

11.3 *Standards*—Prepare calibration standards by dilution of the 1:200 peroxide solution, adding the following volumes (mL) to about 80 mL water in 100 mL volumetric flasks: 0, 0.05, 0.1, 0.25, 0.5, 0.75, 1.5, 3, and 6. Add 2 mL of concentrated derivatizing reagent to each flask, and bring to 100 mL. The resulting solutions represent the following  $H_2O_2$  concentrations ( $\mu M$ ), respectively: 0, 1.5, 2.9, 7.4, 15, 22, 44, 88, and 176. (The actual concentrations in the flasks are one half of these values, but use of the listed values constitutes correction for the 1:1 sample:dilute derivatizing reagent ratio used in the samples.)

NOTE 3—The working stock and working standard solutions above are suggested values, representing the range of concentrations that may be expected in rainwater. Other values for working standard or other ranges of standard concentrations may be required as experience dictates.

## 12. Procedure

12.1 *Analytical System*—The essential component of the analytical system used for peroxide measurement is a fluorometer, which exposes the sample to excitation at 320 nm and measures fluorescent emission at approximately 400 nm. This unit is interfaced with an upstream peristaltic pump and mixing columns that blend reagent solutions to a pH of  $> 10$ , the pH corresponding to optimum intensity of the fluorescence signal. Flow rates are determined by the diameter of the peristaltic tubing selected by the operator. Specific features of the system, including the method of sample introduction (that is, sample injection valve or auto-sampler) and the signal recording device, are chosen on the basis of the sample load and the availability of these resources. The injection valve is used only for manual injection of the sample or standard.

12.2 *Flow System Flushing and Fluorometer Warm-up*—Operate the pump with the NaOH and sample tubes drawing water for a period of time suitable for warm-up and adjustment (according to manufacturer's instructions) of the fluorometer and recording device, normally about  $\frac{1}{2}$  h. Begin flow of the NaOH reagent, and repeat instrument adjustment if necessary.

12.3 *Analysis of Standards, Samples, and Blanks*—Before the introduction of samples, perform a multi-point calibration and check the baseline response of the zero standard and systems blanks. The system is then ready to measure the fluorescence of derivatized samples. Periodically (after the analysis of every 5 to 10 samples), introduce a standard and a blank to provide a measure of system stability. Calculate the concentration of hydroperoxides in samples from the appropriate calibration equation after correcting the fluorescence of derivatized samples for the appropriate measured blank signal (see 12.4 and Section 13).

12.4 *Correction for Intrinsic Peroxides*—The water type specified for this test method may contain concentrations of  $H_2O_2$  sufficient to cause an offset in the analytical baseline and a subsequent bias on the analytical results. If this bias proves to be significant, a correction may be made by preparation and analysis of an intrinsic  $H_2O_2$ -free blank using the  $H_2O_2$ -

destroying enzyme catalase.<sup>3</sup> Add 2 mL of the dilute catalase solution to 100 mL water. Allow 5 min for the  $H_2O_2$  destruction reaction to occur, then immediately prepare a derivatized sample in the same manner as for an atmospheric water sample. Analyze a sufficient quantity of this solution to obtain the intrinsic  $H_2O_2$ -free baseline.

12.4.1 *Removal of Catalase from the Flow System*—If catalase has been introduced into the flow system, it shall be flushed out completely before analysis of  $H_2O_2$  can proceed. Complete removal of catalase can be ensured by flushing for 5 min with 1 M HCl. Follow by flushing for 10 min with water, then successively analyzing  $H_2O_2$  standards until the previously determined fluorescence signal is restored.

## 13. Calculation

13.1 *Linear Response*—A least squares fit relating peak height fluorescence intensity to peroxide (dimer) concentration is calculated from multi-point calibration data. This relationship generally corresponds to a linear least squares equation over the broad concentration range of observed hydroperoxides in precipitation. The linear least squares equation is expressed as

$$y = a + bx \quad (2)$$

where:

$y$  = hydroperoxide concentration,  $\mu M$ ,  
 $x$  = fluorescence intensity, blank corrected,  
 $a$  =  $y$  intercept, and  
 $b$  = slope.

The blank corrected fluorescence ( $x$ ) may be with respect to the calibration blank, the systems blank, or the catalase (see 12.4) blank.

13.2 *Nonlinear Response*—If the fluorescence intensity versus concentration relationship is nonlinear, use a second degree polynomial least squares equation to derive the curve. This equation takes the form:

$$y = ax^2 + bx + c \quad (3)$$

where:

$y$  = hydroperoxide concentration,  $\mu M$ ,  
 $x$  = fluorescence intensity, blank corrected,  
 $c$  =  $y$ -intercept, and  
 $a$  and  $b$  = coefficients of the 1st and 2nd degree variables.


The blank corrected fluorescence ( $x$ ) may be with respect to the calibration blank, the systems blank, or the catalase (see 12.4) blank.

## 14. Precision and Bias

14.1 Because of the instability of hydroperoxides in natural or simulated samples, an inter-laboratory collaborative test for bias and precision was not practical. Single-laboratory determinations of precision yielded 18 % RSD at  $1 \times 10^{-6}$  M and 5 % RSD at  $29 \times 10^{-6}$  M.

## 15. Keywords

15.1 atmospheric water; derivatization; hydrogen peroxide; hydroperoxide; peroxide; sampling

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