



Standard Test Methods for Analysis for Fluoride Content of the Atmosphere and Plant Tissues (Semiautomated Method)¹

This standard is issued under the fixed designation D 3270; 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 These test methods describe the semiautomated procedure for the analyses of various types of samples for the purpose of determining total fluoride. Since the test methods incorporate microdistillation of the sample, they may be applied to any fluoride-containing solution where standards of identical composition have been carried through the same sample preparation procedures and have proven to provide quantitative recovery when analyzed by the semiautomated system. Conversely, the methods shall not be applied for analyses until the applicability has been demonstrated.

1.2 In normal use, the procedure can detect 0.1 $\mu\text{g/mL}$ of F. The normal range of analysis is from 0.1 to 1.6 $\mu\text{g/mL}$ of F. Higher concentrations can be analyzed by careful dilution of samples with reagent water. If digested samples routinely exceed 1.6 $\mu\text{g/mL}$ of F, the analytical portion of the pump manifold can be modified to reduce sensitivity. However, the best procedure is to analyze a smaller aliquot of the sample. Most accurate results are obtained when the fluoride concentration falls in the middle or upper part of the calibration curve.

1.3 The values stated in SI units are to be regarded as the standard. The values given in parentheses are for information only.

1.4 *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 8.3, 10.2.4, and 10.2.5 for additional precautions.

2. Referenced Documents

2.1 ASTM Standards:

D 1193 Specification for Reagent Water²

D 1356 Terminology Relating to Sampling and Analysis of Atmospheres³

¹ These test methods are under the jurisdiction of ASTM Committee D22 on Sampling and Analysis of Atmospheres and are the direct responsibility of Subcommittee D22.03 on Ambient Atmospheres and Source Emissions.

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² *Annual Book of ASTM Standards*, Vol 11.01.

³ *Annual Book of ASTM Standards*, Vol 11.03.

D 3266 Test Method for Automated Separation and Collection of Particulate and Acidic Gaseous Fluoride in the Atmosphere (Double Paper Tape Sampler Method)³

D 3267 Test Method for Separation and Collection of Particulate and Water-Soluble Gaseous Fluorides in the Atmosphere (Filter and Impinger Method)³

D 3268 Test Method for Separation and Collection of Particulate and Gaseous Fluorides in the Atmosphere (Sodium Bicarbonate-Coated Glass Tube and Particulate Filter Method)³

D 3269 Test Methods for Analysis for Fluoride Content of the Atmosphere and Plant Tissues (Manual Procedures)³

D 3614 Guide for Laboratories Engaged in Sampling and Analysis of Atmospheres and Emissions³

3. Terminology

3.1 *Definitions*—For definitions of terms used in these methods, see Terminology D 1356.

4. Summary of Test Methods

4.1 These semiautomated methods are based on the distillation of hydrogen fluoride (HF) from the sample and subsequent reaction of the distillate with alizarin fluorine blue-lanthanum nitrate reagent, to form a blue complex which is measured colorimetrically at 624 nm (**1**)⁴, or the subsequent measurement with a specific ion probe.

4.2 General, Plant Material:

4.2.1 The plant material including leaf samples, washed or unwashed, is dried, and ground, then dissolved with perchloric acid and diluted to 50 mL with water. In the case of leaf samples, an appreciable amount of fluoride may be deposited on the external leaf surfaces. This fluoride behaves differently physiologically from fluoride absorbed into the leaf and it is often desirable to wash it from the surface as a preliminary step in the analysis. Details of a leaf-washing process are described in 9.1.

4.3 General, Atmospheric Samples:

4.3.1 Test Methods D 3269 contains acceptable procedures and also techniques for the proper preparation of atmospheric samples. Test Methods D 3266, D 3267, and D 3268 are

⁴ The boldface numbers in parenthesis refer to the references at the end of this method.

sampling procedures for ambient air and each method contains specific instructions for sample preparation prior to analyses by the semiautomated method.

4.4 General, System Operation:

4.4.1 The dissolved digest is pumped into the polytetrafluoroethylene coil of a microdistillation device maintained at 170°C (2-6). A stream of air carries the acidified sample through a coil of TFE-fluorocarbon tubing to a fractionation column. The fluoride and water vapor distilled from the sample are swept up the fractionation column into a condenser, and the condensate passed into a small collector. Acid and solid material pass through the bottom of the fractionation column and are collected for disposal. Acid and solid material pass through the bottom of the fractionation column and are collected for disposal. In the colorimetric method, the distillate is mixed continuously with alizarin fluorine blue-lanthanum reagent, the colored stream passes through a 15-mm tubular flow cell of a colorimeter, and the absorbance measured at 624 nm. In the potentiometric method, the distillate is mixed continually with a buffer, the mixed streams pass through a flow-through fluoride ion electrode, and the differential millivoltage is measured with an electrometer. The impulse is transmitted to a recorder.

4.4.2 All pieces of apparatus are commercially available, or may be adapted from commercially available equipment. The test method can also be run on most commercially available robot chemical analyzers. Details of construction of the microdistillation device are described in 7.10. Earlier versions of this test method have been published (3,5,6).

4.5 Principle of Operation:

4.5.1 Colorimetric System—The absorbance of an alizarin fluorine blue-lanthanum reagent is changed by very small amounts of inorganic fluoride.

4.5.2 Potentiometric System—Since the sample system is the same for this procedure as for the colorimetric procedure, the distillation step removes all of the interfering cations. The volatile acids that remain can be buffered by mixing with the Total Ionic Strength Adjustment Buffer (TISAB).

4.5.3 Distillation System—Since HF has a high vapor pressure, it is more efficiently distilled than the other acids previously mentioned (4.5). The factors controlling efficiency of distillation are temperature, concentration of acid in the distillation coil, and vacuum in the system. Large amounts of solid matter, particularly silicates, will also retard distillation. Accordingly, the smallest sample of vegetation consistent with obtaining a suitable amount of fluoride should be analyzed. The aforementioned conditions must be carefully controlled, since accurate results depend on obtaining the same degree of efficiency of distillation from samples as from the standard fluoride solutions used for calibration.

4.5.4 Temperature control is maintained within ± 2°C by the thermoregulator and by efficient stirring of the oil bath. Acid concentration during distillation is regulated by taking plant samples in the range from 0.1 to 2.0 g and by using 100 ± 10 mg of CaO and 3.0 ± 0.1 g of NaOH for ashing and fusion of each sample. Vacuum in the system is controlled with flowmeters and a vacuum gage. Any marked change in vacuum (greater than 0.7 kPa or 0.2 in. Hg) over a short time period

indicates either a leak or a block in the system. Distillation should take place at the same vacuum each day unless some other change in the system has been made. It is also essential to maintain the proper ratio between air flow on the line drawing liquid and solid wastes from the distillation coil and on the line drawing HF and water vapor (Fig. 1) from the distillation unit. Occasional adjustments on the two flowmeters should be made to keep this ratio constant and to maintain higher vacuum on the line drawing HF vapor so that little or no HF is diverted into the liquid and solid waste line. (See 10.3.4 for description of air flow system.)

5. Significance and Use

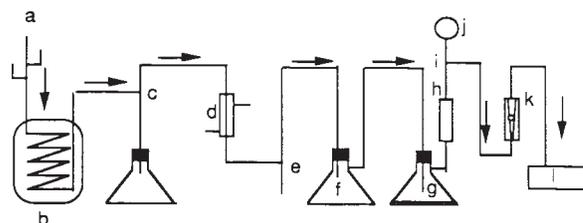
5.1 These test methods may be used for determining the fluoride content of particulate matter and gases collected from the atmosphere by passive and active means, including plant tissues. The user is warned that the fluoride content of passive collectors (including plants) give only qualitative or semiquantitative measurement of atmospheric fluoride content.

6. Interferences

6.1 Since the air that is swept through the microdistillation unit is taken from the ambient atmosphere, airborne contaminants in the laboratory may contaminate samples. If this is a problem, a small drying bulb filled with calcium carbonate granules can be attached to the air inlet tube of the microdistillation unit.

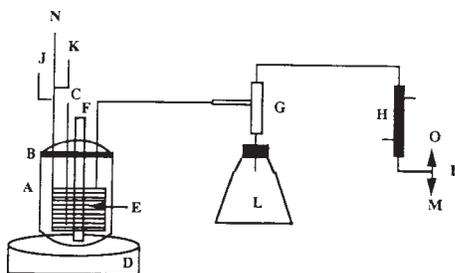
6.2 If the polytetrafluoroethylene distillation coil is not cleaned periodically, particulate matter will accumulate and will reduce sensitivity.

6.3 Silicate, chloride, nitrate, and sulfate ions in high concentration can be distilled with fluoride ion and will interfere with the analysis by bleaching the alizarin fluorine blue-lanthanum reagent. Phosphate ion is not distilled, and therefore does not interfere. Metals such as iron and aluminum are not distilled and will not interfere with the analysis (most materials distilled over do not interfere with the potentiometric method). Maximum concentrations of several common anions at which there was no detectable interference are given in Table 1. The sulfate concentration shown is the amount tolerated above the normal amount of sulfuric acid used in microdistillation. A number of materials cause changes in absorbance at



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|---|--------------------------|---|--|
| a | Air Inlet | g | Waste Bottle with H ₂ SO ₄ |
| b | Microdistillation Coil | h | Gas-Drying Tower |
| c | Fractionation Column | i | T-Tube |
| d | Water-Jacketed Condenser | j | Vacuum Gauge |
| e | Sample Trap | k | Flowmeter |
| f | Waste Bottle | l | Vacuum Pump |

FIG. 1 Schematic Drawing of Air Flow System for Semiautomated Analysis of Fluorides



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|---|---------------------------------|---|-----------------|
| A | Reaction Flask, Flange, Cover | I | Sample Trap |
| B | Flange Clamp | J | Sample Inlet |
| C | Thermometer-Thermoregulator | K | Acid Inlet |
| D | Variable Speed Magnetic Stirrer | L | Waste Trap |
| E | Flexible TFE Tubing | M | Tubing |
| F | Immersion Heater | N | Air Inlet |
| G | Fractionation Column | O | Sample Trap Top |
| H | Water-Jacketed Condenser | | |

FIG. 2 Schematic Drawing of Semiautomated Microdistillation Apparatus

TABLE 1 Maximum Detectable Concentration of Several Anions Present in Samples at Which There is No Detectable Analytical Interference

Compound Tested	Interfering Anion	Molarity Tolerated
Na ₂ SO ₄	SO ₄ (-2)	2 E-2
Na ₂ SiO ₃	SiO ₃ (-2)	5 E-3
NaCl	Cl(-1)	1 E-3
NaH ₂ PO ₄	PO ₄ (-3)	3.8
NaNO ₃	NO ₃ (-1)	5 E-3

624 nm. Potential interfering substances commonly found in plant tissues are metal cations such as iron and aluminum, inorganic anions such as phosphate, chloride, nitrate, and sulfate, and organic anions such as formate and oxalate. Fortunately, metal cations and inorganic phosphate are not distilled in this system, and organic substances are destroyed by preliminary ashing. The remaining volatile inorganic anions may interfere if present in a sufficiently high concentration because they are distilled as acids. Hydrogen ions bleach the reagent which, in addition to being an excellent complexing agent, is also an acid-base indicator. To reduce acidic interferences, a relatively high concentration of acetate buffer is used in the reagent solution despite some reduction in sensitivity.

7. Apparatus

7.1 *Multichannel Proportioning Pump*, with assorted pump tubes, nipple connectors, glass connectors, and manifold platter.

7.2 *Pulse Suppressors*, for the sample and alizarin fluorine blue-lanthanum reagent streams are each made from 3.05 m lengths of 0.89-mm inside diameter polytetra fluoroethylene standard wall tubing. They are both an effective reagent filter and a pulse suppressor. Discard them after one month of use. The outlet ends of the suppressor tubes are forced into short lengths of (0.081-in.) inside diameter silicone rubber tubing, that is then connected to the reagent pump tube, and the other end then slipped over the *h* fitting which joins the sample and reagent streams.

7.3 *Automatic Sampler*, with 8.5 mL plastic sample cups.

7.4 *Voltage Stabilizer*.

7.5 *Colorimeter* (for colorimetric method), with 15-mm tubular flow cell and 624-nm interference filters.

7.6 *Ion Selective Electrode Detector*, (for potentiometric method) with flow-through electrodes.

7.7 *Rotary Vacuum and Pressure Pump*, with continuous oiler.

7.8 *Recorder*.

7.9 *Range Expander*.

7.10 *Microdistillation Apparatus*—A schematic drawing is shown in Fig. 2. Major components of microdistillation apparatus include the following:

7.10.1 *Reaction Flask*, 1000-mL, with a conical flange and cover (Fig. 2 A).

7.10.2 *Reaction Flask Flange Clamp* (Fig. 2B).

7.10.3 *Variable Speed Magnetic Stirrer* (Fig. 2D).

7.10.4 *Thermometer-Thermoregulator*, range 0 to 200°C (Fig. 2 C).

7.10.5 *Electronic Relay Control Box*.

7.10.6 *Immersion Heater*, 500 W (Fig. 2F).

7.10.7 Flexible Polytetrafluoroethylene, TFE tubing, 4.8-mm outside diameter, 0.8-mm wall. A9.14-m length is coiled on a rigid support of such a diameter that the completed coil shall fit into the resin reaction flask (7.10.1). Care must be taken to prevent kinking of the tubing.

7.10.8 *Flowmeter*, with ranges from 0 to 5 L/min, with needle valve control.

7.10.9 *Vacuum Gage*, with a range from 0 to 34 kPa (254 torr).

7.10.10 *Microdistillation Column*, (Fig. 2G, also see Fig. 3).

7.10.11 *Distillate Collector* (Fig. 2I and Fig. 3).

7.10.12 *Water-Jacketed Condenser* (Fig. 2H).

7.10.13 *Heat Exchange Fluid*, for reaction flask (7.10.1).

7.11 *Mechanical Convection Oven*.

7.12 *Wiley Cutting Mill*.

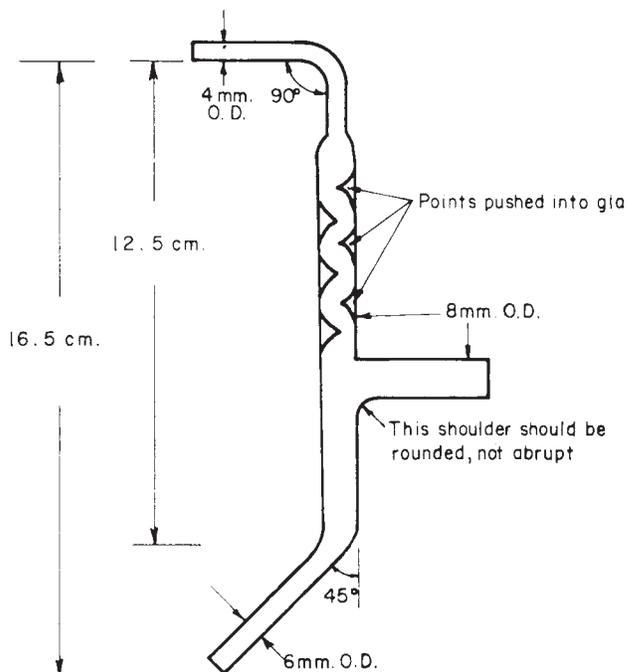


FIG. 3 Microdistillation Column

7.13 *Crucibles*, 40 mL, nickel, platinum or inconel.

7.14 *Muffle Furnace*.

7.15 *pH Meter*.

8. Reagents and Materials

8.1 *Purity of Reagents*—All reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.⁵

8.2 *Purity of Water*—Water shall be Type II reagent water conforming to Specification D 1193

8.3 *Reagents for Automated Fluoride Determination*:

8.3.1 *Acetate Buffer (pH 4.0)*—Dissolve 60 g of sodium acetate trihydrate (8.3.14) in 500 mL of reagent water. Add 100 mL of acetic acid (8.3.2) and dilute to approximately 900 mL with water. Measure the pH and adjust it to pH 4.0 with NaOH (8.3.16.1) or acetic acid (8.3.2). Dilute to 1 L with water.

8.3.2 *Acetic Acid, Glacial*, (sp gr 1.06).

8.3.3 *Acetone*.

8.3.4 *Alizarin Fluorine Blue (alizarin complexone, 3-amino-ethylalizarin-*N,N*-diacetic acid) Stock Solution, 0.01 M*—Suspend 0.9634 g of alizarin fluorine blue in about 100 mL of reagent water in a 250-mL volumetric flask. Add 2 mL of NH₄OH (8.3.5) and shake until dye has completely dissolved. Add 2 mL of glacial acetic acid (8.3.2). Dilute the solution to 250-mL volume with reagent water and store at 4°C.

8.3.5 *Ammonium Hydroxide*, (NH₄OH, sp gr 0.80).

8.3.6 *Wetting Agent*—Dissolve 50 g in 100 mL of reagent water by heating on a hot plate.

8.3.7 **t*-Butanol*.

8.3.8 *CDTA*, (1,2-cyclohexylenedinitrilo)-tetraacetic acid.

8.3.9 *Diluent Solution*—Mix 3.0 mL of 100 ppm standard fluoride solution (8.4.1), 0.5 mL wetting agent (8.3.6) and dilute to 1 L with reagent water.

8.3.10 *ISA Reagent*—Add 2.0 mL wetting agent (8.3.6) to 4.0 L of TISAB (8.3.19) and mix.

8.3.11 *Lanthanum Nitrate Stock Solution (0.01 M)*—Dissolve 2.1652 g of lanthanum nitrate hexahydrate [La(NO₃)₃·6H₂O] in reagent water in a 250-mL volumetric flask and dilute to volume with reagent water.

8.3.12 *Reference Electrode Reagent*—Add 1.0 mL of 100 ppm standard fluoride solution (8.4.1) and 0.5 mL Brij 35 (8.3.6) to a 1-L flask. Dilute to mark with TISAB (8.3.19).

8.3.13 *Silica Gel, Indicating*.

8.3.14 *Sodium Acetate Trihydrate*.

8.3.15 *Sodium Chloride* (NaCl).

8.3.16 *Sodium Hydroxide Pellets* (NaOH).

8.3.16.1 *Sodium Hydroxide Solution, (5 N)*—Dissolve 220 g NaOH in reagent water, and dilute to 1 L.

8.3.17 *Sulfuric Acid, Concentrated*, (sp gr 1.84) (H₂SO₄).

8.3.18 *Sulfuric Acid, Analytical Grade, (50 %, volume per volume)*—Mix 500 mL of H₂SO₄ (8.3.17) with 500 mL of reagent water. Cool before use. (**Warning**—This mixture can react violently. Use eye protection and prepare in safety hood.)

8.3.19 *TISAB Buffer*—Add 57 mL glacial acetic acid (8.3.2), 58 g NaCl (8.3.15), and 4.0 g CDTA (8.3.8) to 500 mL of reagent water. Stir to dissolve and add 5 N NaOH (8.3.16.1) until pH is between 5.0 and 5.5. Cool and dilute to 1 L.

8.3.20 *Alizarin Fluoride Blue-Lanthanum Reagent*—The reagent is a modification of that reported by Yamamura, et al (7). Mix the following quantities of solutions in the order listed to make 1 L of alizarin blue-lanthanum reagent: 300 mL of acetate buffer (8.3.1), 244 mL of reagent water, 300 mL of acetone (8.3.3), 100 mL *t*-butanol (8.3.7), 36 mL of alizarin fluorine blue (8.3.4), 20 mL of La(NO₃)₃·6H₂O (8.3.11), and 40 drops of wetting agent (8.3.8). Just prior to using reagent, place under vacuum for about 10 min to remove dissolved air. Unused working reagent is stable at 4°C for at least 7 days.

8.4 *Standards*.

8.4.1 *Standard Fluoride Solutions*—Dissolve 0.2207 g of dry reagent grade NaF (that had been stored in a desiccator prior to use) in reagent water and dilute to 1 L. The stock solution will contain 100 µg F/mL (100 ppm).

8.4.1.1 *Sodium Fluoride Solution (10 ppm)*—Dilute 100 mL of the 100 ppm NaF solution (8.4.1) to 1 L.

8.4.2 *Working Standards*—Prepare working standards by taking suitable aliquots to eight final concentrations of 0.2, 0.4, 0.8, 1.0, 1.6, 2.4, 3.2, and 4.0 µg F/mL. Plant analysis shall contain 6 g NaOH (8.3.16) and 20 mL HClO₄ (8.4.3) for each 100 mL of solution in order to compensate for the amounts of these substances used in alkali fusion of the ashed plant samples. Standard solutions for fluoride analysis of water samples or of air samples absorbed in water are made up in reagent water. Store stock solutions in clean polyethylene bottles in the cold. Since, as will be seen later, plant tissue samples are diluted to a 50-mL volume before analysis, the standard containing 0.20 µg/mL of fluoride is equivalent to a sample of plant material containing 10.0 µg of fluoride (0.20 µg/mL × 50 mL).

8.4.3 *Perchloric Acid, concentrated, (70 %)* (HClO₄, sp gr 1.66).

8.4.3.1 *Perchloric Acid, (1 + 1 Solution)*—Dilute 250 mL of concentrated HClO₄ (8.4.3) to 500 mL with reagent water.

8.5 *Tetrasodium Ethylenediamine Tetraacetate (Na₄EDTA)*, 1 % (weight per volume)—Dissolve 1 g of Na₄EDTA in 99 mL of reagent water.

8.6 *Plant Tissue Solution*—Dissolve 0.5 g of detergent and 0.5 g Na₄EDTA in reagent water to make 1 L.

8.7 *Reagents for Ashing and Alkali Fusion of Plant Samples*:

8.7.1 *Calcium Oxide* (CaO), with a known, low fluoride content.

8.7.2 *Cheesecloth*.

8.7.3 *Ethanol or Propanol*.

8.7.4 *Hydrochloric Acid, (4 N)*—Dilute 342 mL of concentrated hydrochloric acid (HCl, sp gr 1.19) to 1 L.

8.7.5 *Kraft Paper Bags*.

⁵ *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.

8.7.6 *Phenolphthalein Solution (1 %)*—Dissolve 1 g phenolphthalein in 50 mL of absolute ethanol or isopropanol. Add 50 mL of reagent water.

8.7.7 *Polypropylene Bags*, with moisture seal.

8.7.8 *Sodium Hydroxide Solution (10 %)*—Dissolve 10 g of NaOH (8.3.16) in water, cool, and dilute to 100 mL.

8.8 *Effect of Storage*:

8.8.1 The acetate buffer (8.3.1) and Brij 35 (8.3.6) solutions are stable at room temperature. Stock solutions of alizarin fluorine blue (8.3.4) and lanthanum nitrate (8.3.11) are stable indefinitely at 4°C. The alizarin fluorine blue-lanthanum working reagent (8.3.11) is stable at 4°C for at least 7 days. Diluted NaF solutions (8.4.1 and 8.4.2) shall be stored in the cold in polyethylene bottles and are stable in the presence of NaOH. Tightly covered ashed and fused plant samples appear to be stable indefinitely. All reagents except for the concentrated acids and alkalis, and the organic solvents, may be stored in a laboratory refrigerator. Warm the solutions to room temperature before use.

9. Calibration and Standardization

9.1 Transfer portions of each calibration fluoride solution (0.2, 0.4, 0.8, 1.0, 1.6, 2.4, 3.2, and 4.0 µg F/mL) to 8.5-mL sample cups. Place the calibration sample cups through the sample tray in a random order. Proceed with the analysis as described in 10.3. A calibration curve should precede and follow each day's set of samples.

9.2 After calibration solutions have been analyzed and the peaks plotted by the chart recorder, draw a straight line connecting the baseline before and after the analysis. Record the absorbance of each peak and subtract the absorbance of the baseline. Compute the regression of net absorbance versus µg/mL of fluoride by the method of least squares.

10. Procedure

10.1 *Preparation of Plant Tissues for Analysis*:

10.1.1 This procedure is used to remove fluoride from surface tissues without altering the internal concentration of fluoride. Whether vegetation samples are to be washed will depend on the intended use of the population of plants the sample represents. For example, in foliage crops or other vegetation intended for consumption by herbivores, fluoride on foliar surfaces as well as that within leaves is important and should be included in the analysis. For other kinds of vegetation, fluoride deposited on the surface of leaves may be unimportant with respect to the plant, and it may be desirable to wash it from the surface prior to analysis.

10.1.2 *Criteria*—A standard washing procedure should meet several washing criteria: it should be simple and gentle; it should remove surface fluoride quantitatively with a minimum of leaching of internal fluoride; it should not leave residues that might interfere with subsequent analysis; and, in the event that the tissue is to be analyzed also for nutrient status, it should not leach other internal elements. These criteria and methods are discussed thoroughly in the literature (6, 8-21).

10.1.3 *Procedure*—Fresh tissues are placed in a cheesecloth square, the ends of the square folded up, and the whole washed in a polyethylene container filled with the plant tissue wash

solution (8.6) for 30 s with gentle agitation. The cheesecloth containing the tissue is removed and allowed to drain for a few s, and then rinsed for 10 s in each of three containers of reagent water. Dry the tissue by blotting it with dry paper towels.

10.1.3.1 The washed and partially dried tissue is then placed in a labeled Kraft paper bag (8.7.5) and dried in the mechanical convection oven at 80°C for no less than 24 h.

10.1.3.2 Grind dried tissues in a semimicro Wiley mill to pass a 40-mesh sieve. The sieved material is collected and placed in a polyethylene container with a moisture seal (8.7.7).

10.2 *Procedure for Ashing and Alkali Fusion of Plant Tissues*:

10.2.1 Mix the dried sample thoroughly and carefully weigh from 0.1 to 1.0 g of plant tissue, depending on the fluoride content, into a clean crucible.

10.2.2 Add 100 ± 10 mg of CaO (8.7.1), sufficient reagent water to make a loose slurry, and 2 drops of phenolphthalein (8.7.6). Mix thoroughly with a polyethylene policeman. The final mixture will be uniformly red in color and will remain red during evaporation to dryness.

10.2.3 Place crucibles on a hot plate and under infrared lamps. Turn on infrared lamps (do not turn on hot plate) until all liquid is evaporated. Turn on hot plate and char samples for 1 h.

10.2.4 Transfer crucibles to a muffle furnace at 600°C and ash for 2 h. (**Warning**—To avoid flaming, place crucibles at front of muffle furnace with door open for about 5 min to further char samples. Then position crucibles in the furnace.)

10.2.5 After ashing, remove crucibles (not more than eight at one time), add 3 ± 0.1 g of NaOs pellets (8.3.16), and replace in the furnace with door closed for 3 min. (**Warning**—Watch out for creeping of the molten NaOH. Discard the sample if it has crept from the crucible.) Remove crucibles one at a time and swirl to suspend all particulate matter until the melt is partially solidified. Allow crucible to cool until addition of small amount of water does not cause spattering. Wash down inner walls of crucible with 10 to 15 mL of reagent water.

10.2.6 After crucibles have cooled to room temperature, stir the melt with a polyethylene policeman and transfer to a 50.0-mL plastic cylinder, using reagent water. Rinse crucible with 20.0 mL of a 1 + 1 70 % HClO₄ (8.4.3.1) and add to the tube. Make sample to 50.0-mL volume with water.

10.2.7 Run several blank crucibles (about one blank for each 10 samples) containing all reagents through the entire procedure.

10.2.8 *Cleaning of Crucibles*—Clean the crucibles as soon as possible after use. Soak inconel crucibles in 10 % NaOH (8.7.7) overnight. Follow by washing with hot water and scouring with a soap-free steel wool pad. Rinse three times with water followed by three rinses with reagent water. Then immerse them in 4 N HCl (8.7.4) for 1.5 h, rinse them three times in tap water followed by three times in reagent water. Perform a blank analysis on the crucibles before use to check on contamination.

10.3 *Procedure for Automated Analysis of Samples*:

10.3.1 *Distillation*—Refer to Figs. 1-3. All flow rates given are nominal values. Standard fluoride solutions, ashed and alkali-fused samples, or impinged air samples are placed in

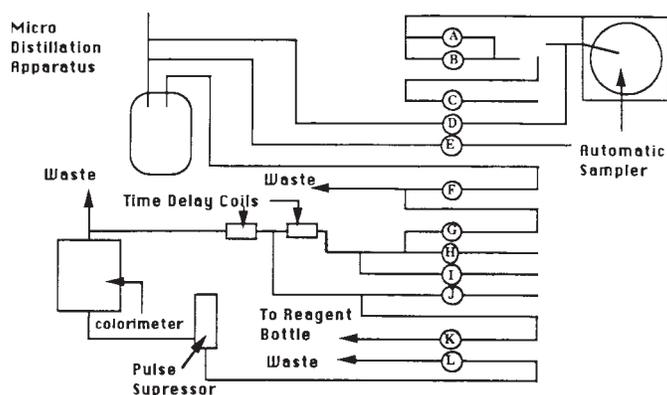
8.5-mL plastic cups in the sampler module. The sampler is actuated, and the sample is pumped from the cup at a net rate of 3.48 mL/min with air segmentation of 0.42 mL/min after the sampler crook (3.90 mL-0.42 mL = 3.48 mL) and is pumped into the microdistillation device through the sample inlet (Fig. 2J). Fifty % H₂SO₄(8.3.18) is pumped at 2.50 mL/min through the acid inlet (Fig. 2K). Acid and ashed solids drop into the waste flask (Fig. 2I) and are discarded after the run. Distillate is pumped from the sample trap at 2.50 mL/min through 1.35-mm inside diameter polytetrafluoroethylene tubing (Fig. 2M), and air segmented with 0.42 mL/min air. Air enters the system at the inlet (Fig. 2N) and leaves the system at the top of the sample trap (Fig. 2O).

10.3.2 *Colorimetric Analysis*—See Fig. 4. The sample stream is then resampled at 0.32 mL/min and the remainder of the sample stream goes to waste. The resampled stream is mixed with diluent solution (8.3.9) at 0.80 mL/min and air segmented at 0.42 mL/min. The mixed stream passes through a polychlorotrifluoro-ethylene mixing coil and alizarin fluoride blue lanthanum reagent (8.3.4) is added at 0.97 mL/min by pumping the reagent with a 1.20 mL/min silicone tubing and resampling through a 0.23 mL/min silicone tubing. Pass the alizarin fluoride blue-lanthanum reagent through an inline filter prior to mixing with the sample stream. The two liquid streams are combined and passed through a second polychlorotrifluoroethylene coil for time delay, proper mixing, and color development. The reagent stream then passes through a debubbler fitting where a small portion of the sample stream is removed (along with any air bubbles) and passes to waste. The remainder of the sample stream passes through a 15-mm tubular flow cell of the colorimeter, and the absorbance is measured at 624 nm. The sample stream is drawn through the flow cell and a glass pulse suppressor with a silicone tubing at 1.40 mL/min. Results are plotted on a chart recorder. The lag time from sampling to the appearance of a peak on the chart recorder is about 5 min.

10.3.3 *Potentiometric Analysis*—See Fig. 5. The sample stream (from 10.3.1) is then resampled at 1.20 mL/min and the remainder of the sample stream goes to waste. The resampled stream is mixed with ISA Reagent (8.3.10) at 0.80 mL/min, and air segmented (0.42 mL/min). The mixed stream passes through a polychlorotrifluoro-ethylene mixing coil to insure proper mixing. The mixed stream then passes through a debubbler fitting and the air and excess sample pass to waste. The debubbled sample stream then passes through the flow-through fluoride electrode assembly. The reference electrode reagent (8.3.12) is pumped at 0.10 mL/min and passed through the flow-through reference fluoride electrode. The waste from both electrodes are sampled at 0.80 mL/min and pumped to waste.

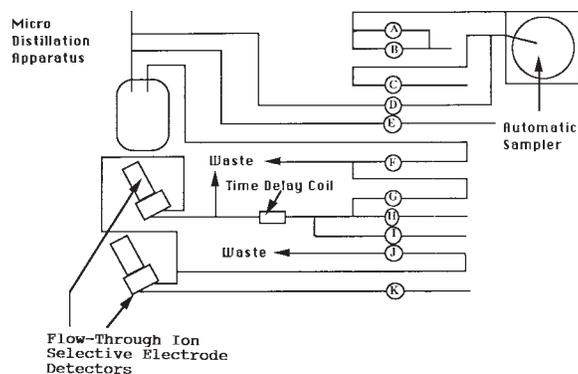
10.3.4 *Description of Air Flow System (Refer to Fig. 1)*—Air is drawn through the air inlet tube (a) before the polytetrafluoroethylene microdistillation coil (b). Air sweeps through (b) to the fractionation column (c) where the air stream is diverted through the water-jacketed condenser (d) and sample trap (e) to waste bottle (f). The air then passes through a 3.2-mm inside diameter glass tube directed against the surface of concentrated H₂SO₄ (8.3.17) contained in waste bottle (g). The partially dehydrated air passes through a gas-drying tower (h) containing 450 g of indicating silica gel (8.3.13). Air leaving the outlet of the drying tower passes through a T-tube (i) to which a vacuum gage (j) (0 to 34 kPa) is connected, through a flow meter (k) (0 to 5 L/min), and then to the vacuum pump (l).

10.3.5 *Start-Up Procedures*—Turn on the water to condenser. Turn on the colorimeter. Engage the manifold on the proportioning pump and start the pump. Turn on the vacuum pump and set flow rates. Turn on the stirring motor in the microdistillation unit. Connect the lines to the H₂SO₄ solution (8.3.18), to the alizarin blue-lanthanum reagent (8.3.20), to the diluent solution (8.3.9) and to the reagent water bottles. In the case of the potentiometric method, connect the lines to the



Tube	Reagent	Flow Rate mL/min	Tube	Reagent	Flow Rate mL/min
A	Water	3.90	G	Sample	0.32
B	Water	1.60	H	Air	0.42
C	Air	0.42	I	Diluent (8.3.9)	0.80
D	Sample	3.90	J	Alizarin (8.3.20)	1.20
E	H ₂ SO ₄ (8.3.18)	2.50	K	Repump	0.23
F	Resample	2.50	L	Waste	1.40

FIG. 4 Semiautomated Colorimetric Method



Tube	Reagent	Flow Rate mL/min	Tube	Reagent	Flow Rate mL/min
A	Water	3.90	G	Sample	1.20
B	Water	1.60	H	Air	0.42
C	Air	0.42	I	ISA (8.3.10)	0.80
D	Sample	3.90	J	Electrode Waste	0.80
E	H ₂ SO ₄ (8.3.18)	2.50	K	Ref Electr (8.3.12)	0.10
F	Resample	2.50			

FIG. 5 Semiautomated Potentiometric Method

TISAB (8.3.19) and reference electrode reagent (8.3.12). Place the sampling tube of the sampler unit in the water reservoir. Allow the apparatus to equilibrate until the oil bath in the microdistillation unit has reached 170°C. Be sure that all tubing connections are secure. Adjust flowmeter that controls distillation (Fig. 1k) to 4 L/min. Distillate should now fill the sample trap. Readjust the flowmeter (Fig. 1k) to give a reading on the vacuum gage of 17 to 20 kPa (127 to 150 torr). Determine satisfactory setting for each instrument by trial and error. Once a satisfactory value is determined, it is important that this setting be maintained each day. No air bubbles should be in the analytical system beyond the point where the alizarin fluorine blue-lanthanum reagent and distillate streams are joined. Turn on the chart recorder, adjust the baseline to the desired level, and run a baseline for several minutes to assure that all components are operating properly. Transfer standard fluoride solutions to 8.5-mL plastic cups and place in sampler. Separate the last standard sample from unknown samples with one cup containing reagent water. Program the sampler for 20 samples/h with 1:3 sample to wash ratio. Turn the sampler on. Analyze calibration solutions before and after each day's set of samples.

10.3.6 Shut-Down Procedure—Turn off the chart recorder. Disconnect the H₂SO₄ reagent (8.3.18) line and place in reagent water. In the case of the colorimetric procedure, disconnect the alizarin fluorine blue-lanthanum reagent (8.3.20) line and place in 1 % EDTA solution for about 1 min. Transfer the line to reagent water and allow water to pass through the analytic system for about 5 min, or until no color remains in lines. In the case of the potentiometric method, disconnect the resample line and pump 10 mg/L fluoride (8.4.2.1) into the sample stream for 5 min. Then clamp off the waste lines before lifting off the pump platen. Clean out TFE tubing (7.10.7) distillation coil by allowing 10 to 20 mL of 4 N HCl (8.7.4) to be drawn through the air inlet tube and into the coil. Follow this by a thorough rinse with reagent water. Turn

off the heater and stirring motor in the microdistillation unit. Turn off the vacuum pump. Release pump tube manifold. Turn off water to the condensers.

10.3.7 Maintenance:

10.3.7.1 Replace the pump tubes after 200 working h or prior to that if they become hard and inflexible or flattened.

10.3.7.2 Regenerate the indicating silica gel (8.3.13) when two thirds of the amount in each container loses its normal blue color.

10.3.7.3 Oil the proportioning pumps once a month, and check and adjust the recorder gain monthly.

10.3.7.4 Clean all tubing containing reagents after each daily run with Na₄EDTA solution followed by reagent water.

10.3.7.5 Leave pump tubes in the relaxed position when not in use.

10.3.8 Check Procedures:

10.3.8.1 Always make an estimate of the degree of fluoride contamination from reagents and equipment. Carry crucibles without sample but with all reagents through the entire procedure due to the ubiquity of fluoride. Contamination from previously analyzed samples, from a contaminated muffle furnace, and from reagents can then be detected and corrective efforts made. Blank values over 5 µg F are considered evidence of contamination. Make two blank determinations with every batch of samples analyzed (approximately 2 blanks for 40 samples).

10.3.8.2 Occasionally prepare a special calibration curve by adding known amounts of NaF solution from a microburet to aliquots of a low-F tissue. Recovery of added fluoride should be 100 ± 10 %. Low values indicate loss of fluoride, possibly during pretreatment, and high values indicate contamination.

10.3.8.3 Occasionally analyze different amounts (0.1 to 1.0 g) of a plant sample containing 50 to 65 ppm fluoride. A linear relationship should exist between fluoride found and amounts of tissue taken. A nonlinear relationship may indicate that some

component of the tissue is retarding distillation or interfering with color development.

10.3.8.4 Repeat calibration curves at least twice daily to correct for any small changes in distillation efficiency that might occur.

10.3.8.5 If the sample-to-sample absorption ratio exceeds 5:1, then place a water blank sample between the high and low samples.

10.3.9 *Troubleshooting*—Since no method for determining μg quantities in an overwhelming excess of other materials is free of occasional problems, suggestions on how to recognize the difficulties and systematically locate the problems may be of value. Fortunately, most of the potential problems in distillation and fluoride analysis are manifested by obvious irregularities on the chart recorder.

10.3.9.1 Irregular fluctuations in the baseline may result from the following: excessive surge pressures in the liquid streams; air bubbles passing through the flow cell in the photometer; or bleaching of the reagent by excess sulfuric acid carryover during distillation or insufficient buffer in the reagent. Excessive pulse pressures may be due to faulty pump tubes, the absence of surge suppressors, or the presence of surge suppressors that are improperly made or placed. Air bubbles in the photometer flow cell may be due to the absence of a debubbler bypass, a blockage in the reagent pump tube, or a periodic emptying of the sample trap. The last condition will result if the air flow to the distillation trap becomes too great. Excessive sulfuric acid carryover can be caused by too high a temperature in the oil bath, improper sulfuric acid concentration, or too high a vacuum on the system. Large fluctuations or imbalances in vacuum or air flow rates in the distillation or waste systems will also produce baseline irregularities. An improper flowmeter setting, trapped air in the tubing, or a leak or block in the system should be sought as the probable cause of this type of difficulty.

10.3.9.2 Asymmetrical peaks, double peaks, or peaks with shoulders may result from: baseline irregularities, interfering substances from the sample or impure reagents, inadequate buffer concentration, or excessive amounts of solid material in the distillation coil. The presence or accumulation of excessive solids may be due to insufficient flow of H_2SO_4 , too large a sample, excessive amounts of calcium oxide or sodium hydroxide in the sample, inadequate suspension of particles in the samples, or lack of proper air segmentation in the sample tubing.

10.3.9.3 Poor reproducibility can be caused by improper sample pickup; by faulty pump tubes; by inadequate washout of the distillation coils between samples; by large deviations in acid concentration temperature, or air flow in the distillation coil; or by changes in vacuum on the waste systems.

11. Calculation

11.1 Calculate the fluoride content of the sample as follows:

$$F_T = F_S V_S D / W_S \quad (1)$$

where:

F_T = concentration of fluoride in sample, ppm(v),

F_S = concentration of fluoride in unknown sample as taken from the calibration curve, $\mu\text{g}/\text{mL}$,

V_S = volume of the unknown sample, usually 50 mL,

D = dilution factor used when fluoride in unknown sample exceeds the standard curve. For example, if the original sample is diluted from 50 mL to 100 mL, D will equal 2, and

W_S = mass of sample taken for analysis, g.

NOTE 1—All dilutions of plant samples should be made with a solution containing 6 g of NaOH and 40 mL of 1 + 1 70 % HClO_4 (8.4.3.1) per 100 mL. If the unknown sample is not diluted, drop D from the calculations.

11.2 *Atmospheric Samples*—Determine the concentration of fluoride in $\mu\text{g}/\text{mL}$ from the calibration curve generated in 9.2. Calculate the atmospheric fluoride concentration by calculating the amount of fluoride in the original air sample, and dividing by the volume of air sampled, corrected to standard conditions, as follows:

$$F_a = (F_S V_S) 1000 / V_a \quad (2)$$

where:

F_a = fluoride concentration in the atmosphere, $\mu\text{g}/\text{m}^3$,

F_S = fluoride concentration in the sample solution, $\mu\text{g}/\text{mL}$,

V_S = volume of air sample, corrected to 101.3 kPa (760 mm of Hg) and 25°C, and

1000 = factor to convert L to m^3 .

12. Precision and Bias

12.1 *General*—It is essential that each laboratory periodically perform the tests outlined above (10.3.8.1) according to the laboratory's Quality Assurance Plan. See Guide D 3614 for guidelines for establishing the QA Plan. The degree of precision and bias obtained in one laboratory may not be a fair representation for another laboratory, since much depends on the thoroughness with which any multi-step analytical procedure is carried out. The wide variations in results obtained in a cooperative study among 31 laboratories (12) emphasizes the importance of proper performance methods. The following information on precision and bias is meant, therefore, as a guide and is taken from a published report (2) in which results of analyses of tissues by the Willard-Winter (20,21) methods were compared.

12.2 *Precision*—The standard error of a single determination is between 2 and 8 μg , depending on the plant tissue used, and the level of fluoride present. With higher amounts of fluoride, the standard deviation increases although the coefficient of variation (standard deviation expressed as a percentage of fluoride content) decreases. The coefficient of variation between 20 and 100 ppm(v) fluoride is generally less than 10 %. Analysis of high silicate tissues (grasses) for fluoride has always been a problem and standard deviations of results of replicate determinations on orchard grass tissues have been larger than when other tissues have been analyzed. Recent modifications in procedures for transferring and distilling samples have improved analysis of grasses.

12.3 *Bias*—Since no direct means of determining bias for this method have yet been established, indirect means have been employed. A total of 180 determinations of four tissues

(Milo maize, gladiolus, alfalfa, and orchard grass) were performed. Within the limits of reproducibility of the results, multiple linear regression analysis indicated that no significant deviations from linearity were obtained when different amounts of fluoride were added to a tissue. In addition, there were no significant second-order effects. Systematic errors

were not significant since the intercept values found (-3.97 to $+2.17 \mu\text{g}$) were not significantly different from zero.

13. Keywords

13.1 ambient atmospheres; colorimetric determination of fluoride; fluoride; potentiometric determination of fluoride

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