Standard Test Method for Determination of Dissolved Inorganic Anions in Aqueous Matrices Using Capillary Ion Electrophoresis and Chromate Electrolyte[1]

This standard is issued under the fixed designation D 6508; 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 cover the determination of the inorganic anions fluoride, bromide, chloride, nitrite, nitrate, orthophosphate, and sulfate in drinking water, wastewater, and other aqueous matrices using capillary ion electrophoresis (CIE) with indirect UV detection. See Figs. 1-6.

1.2 The test method uses a chromate-based electrolyte and indirect UV detection at 254 nm. It is applicable for the determination or inorganic anions in the range of 0.1 to 50 mg/L except for fluoride whose range is 0.1 to 25 mg/L.

1.3 It is the responsibility of the user to ensure the validity of this test method for other anion concentrations and untested aqueous matrices.

NOTE 1—The highest accepted anion concentration submitted for precision and bias extend the anion concentration range for the following anions: Chloride to 93 mg/L, Sulfate to 90 mg/L, Nitrate to 72 mg/L, and ortho-phosphate to 58 mg/L.

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.* For specific hazard statements, see Section 9.

2. Referenced Documents

2.1 *ASTM Standards:*

D 1066 Practice for Sampling Steam[2]
D 1129 Terminology Relating to Water[2]
D 1193 Specification for Reagent Water[2]
D 2777 Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D-19 on Water[2]
D 3370 Practices for Sampling Water from Closed Conduits[2]
D 3856 Guide for Good Laboratory Practices in Laboratories Engaged in Sampling and Analysis of Water[2]
D 5810 Guide for Spiking into Aqueous Samples[2]
D 5847 Practice for Writing Quality Control Specifications for Standard Test Methods for Water Analysis[3]

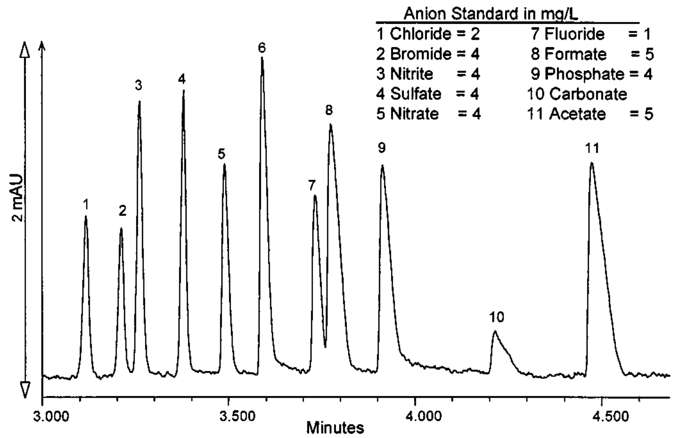

FIG. 1 Electropherogram of Mixed Anion Working Solution and Added Common Organic Acids

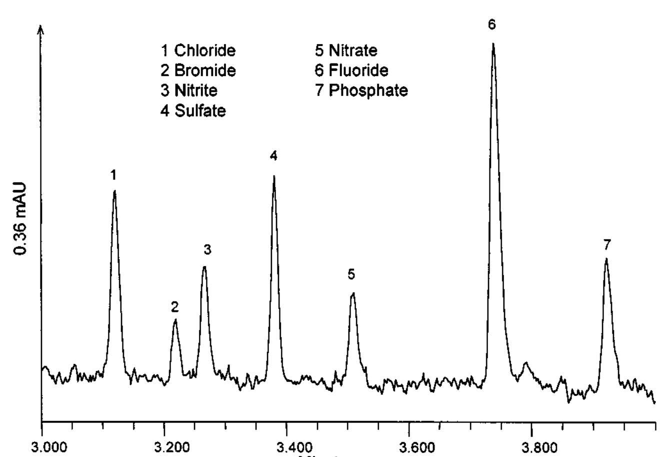

FIG. 2 Electropherogram of 0.2 mg/L Anions Used to Determine MDL

[1] This test method is under the jurisdiction of ASTM Committee D-19 on Water and is the direct responsibility of Subcommittee D19.05 on Inorganic Constituents in Water.

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

[3] *Annual Book of ASTM Standards*, Vol 11.02.

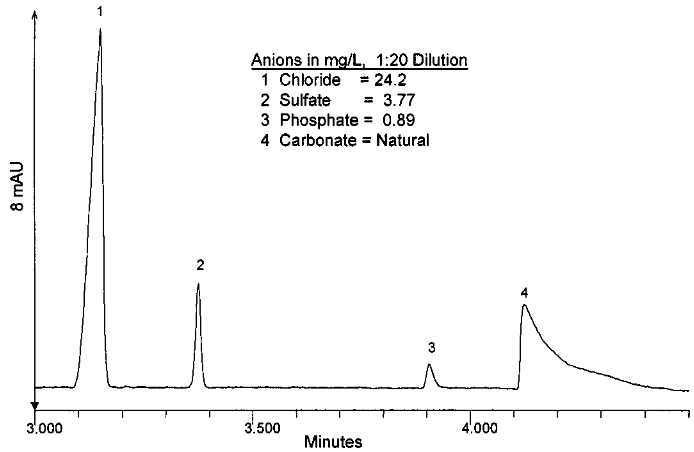

FIG. 3 Electropherogram of Substitute Wastewater

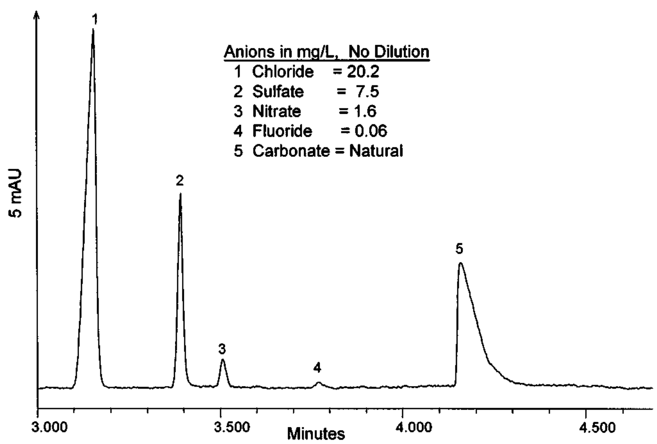

FIG. 4 Electropherogram of Drinking Water

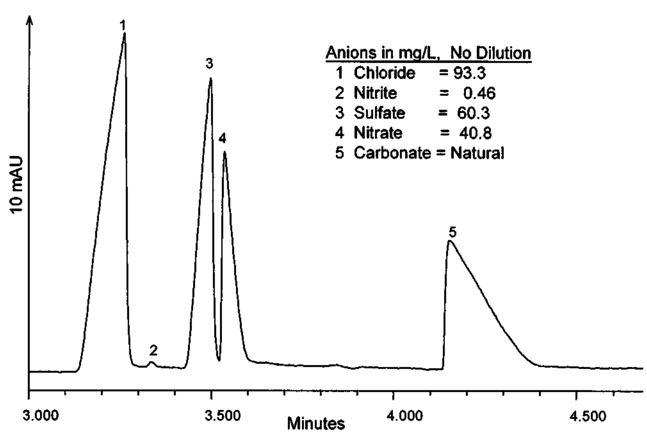

FIG. 5 Electropherogram of Municipal Wastewater Treatment Plant Discharge

D 5905 Practice for the Preparation of Substitute Wastewater[2]

F 488 Test Method for On-Site Screening of Heterotrophic Bacteria in Water[3]

3. Terminology

3.1 *Definitions*—For definitions of terms used in this test method, refer to Terminology D 1129.

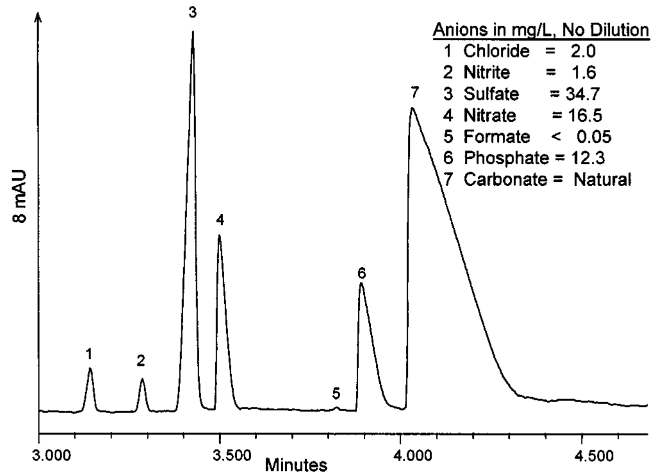

FIG. 6 Electropherogram of Industrial Wastewater

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *capillary ion electrophoresis*, *n*—an electrophoretic technique in which a UV-absorbing electrolyte is placed in a 50 µm to 75 µm fused silica capillary. Voltage is applied across the capillary causing electrolyte and anions to migrate towards the anode and through the capillary's UV detector window. Anions are separated based upon the their differential rates of migration in the electrical field. Anion detection and quantitation are based upon the principles of indirect UV detection.

3.2.2 *electrolyte*, *n*—a combination of a UV-absorbing salt and an electroosmotic flow modifier placed inside the capillary, used as a carrier for the analytes, and for detection and quantitation. The UV-absorbing portion of the salt must be anionic and have an electrophoretic mobility similar to the analyte anions of interest.

3.2.3 *electroosmotic flow (EOF)*, *n*—the direction and velocity of electrolyte solution flow within the capillary under an applied electrical potential (voltage); the velocity and direction of flow is determined by electrolyte chemistry, capillary wall chemistry, and applied voltage.

3.2.4 *electroosmotic flow modifier (OFM)*, *n*—a cationic quaternary amine in the electrolyte that dynamically coats the negatively charged silica wall giving it a net positive charge. This reverses the direction of the electrolyte's natural electroosmotic flow and directs it towards the anode and detector. This modifier augments anion migration and enhances speed of analysis. Its concentration secondarily effects anion selectivity and resolution, (see Fig. 7).

3.2.5 *electrophoretic mobility*, *n*—the specific velocity of a charged analyte in the electrolyte under specific electroosmotic flow conditions. The mobility of an analyte is directly related to the analyte's equivalent ionic conductance and applied voltage, and is the primary mechanism of separation.

3.2.6 *electropherogram*, *n*—a graphical presentation of UV-detector response versus time of analysis; the x axis is migration time, which is used to qualitatively identify the anion, and the y axis is UV response, which can be converted to time corrected peak area for quantitation.

3.2.7 *hydrostatic sampling*, *n*—a sample introduction technique in which the capillary with electrolyte is immersed in the sample, and both are elevated to a specific height, typically 10

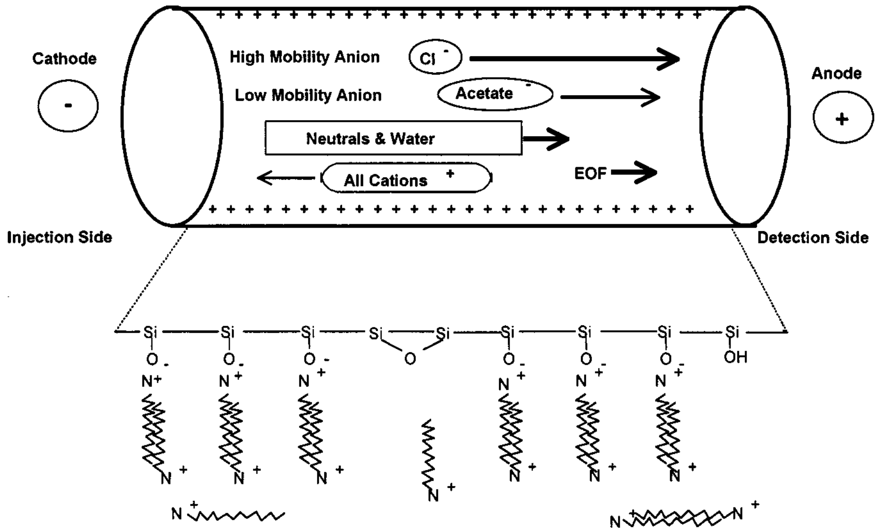

FIG. 7 Pictorial Diagram of Anion Mobility and ElectroOsomotic Flow Modifier

cm, above the receiving electrolyte reservoir for a preset amount of time, typically less than 60 s. Nanolitres of sample are siphoned into the capillary by differential head pressure and gravity.

3.2.8 *indirect UV detection*, *n*—a form of UV detection in which the analyte displaces an equivalent net charge amount of the highly UV-absorbing component of the electrolyte causing a net decrease in background absorbance. The magnitude of the decreased absorbance is directly proportional to analyte concentration. Detector output polarity is reversed in order to obtain a positive mV response.

3.2.9 *midpoint of peak width*, *n*—CIE peaks typically are asymmetrical with the peak apex shifting with increasing concentration, and the peak apex may not be indicative of true analyte migration time. Midpoint of peak width is the midpoint between the analyte peak's start and stop integration, or the peak center of gravity.

3.2.10 *migration time*, *n*—the time required for a specific analyte to migrate through the capillary to the detector. The migration time in capillary ion electrophoresis is analogous to retention time in chromatography.

3.2.11 *time corrected peak area*, *n*—normalized peak area; peak area divided by migration time. CE principles state that peak area is dependent upon migration time, that is, for the same concentration of analyte, as migration time increases (decreases) peak area increases (decreases). Time corrected peak area accounts for these changes.

4. Summary of Test Method

4.1 Capillary ion electrophoresis, see Figs. 7-10, is a free zone electrophoretic technique optimized for the determination of anions with molecular weight less than 200. The anions migrate and are separated according to their mobility in the electrolyte when an electrical field is applied through the open tubular fused silica capillary. The electrolyte's electroosmotic low modifier dynamically coats the inner wall of the capillary changing the surface to a net positive charge. This reversal of wall charge reverses the natural EOF. The modified EOF in combination with a negative power supply augments the mobility of the analyte anions towards the anode and detector achieving rapid analysis times. Cations migrate in the opposite direction towards the cathode and are removed from the sample during analysis. Water and other neutral species move toward

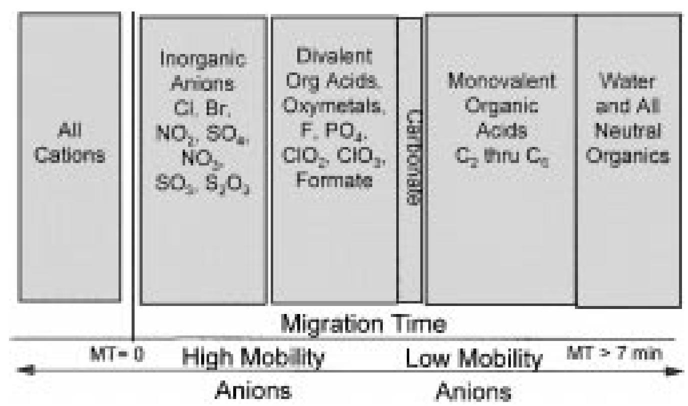

FIG. 8 Selectivity Diagram of Anion Mobility Using Capillary Ion Electrophoresis

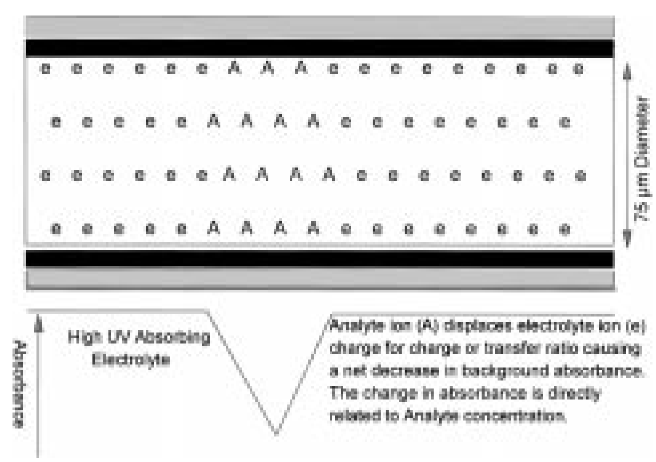

FIG. 9 Pictorial Diagram of Indirect UV Detection

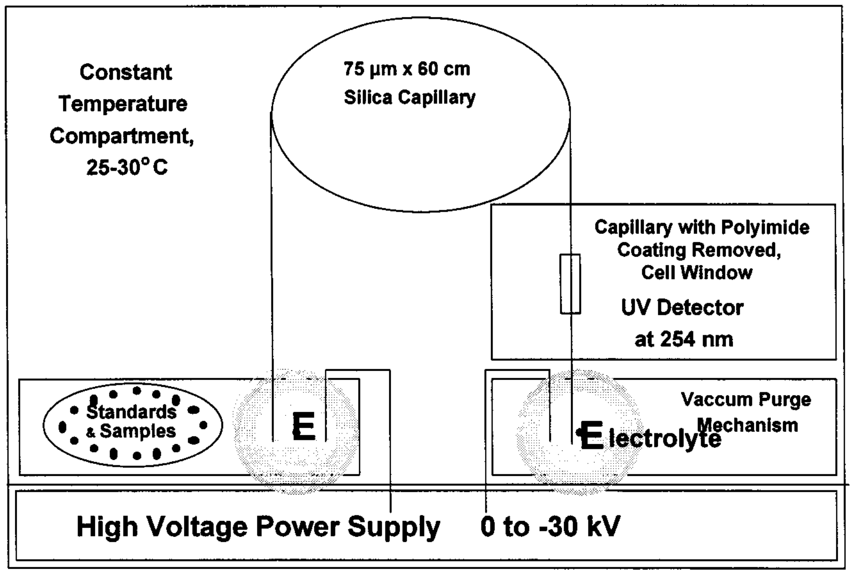

FIG. 10 General Hardware Schematic of a Capillary Ion Electrophoresis System

the detector at the same rate as the EOF. The neutral species migrate slower than the analyte anions and do not interfere with anion analysis (see Figs. 7 and 8).

4.2 The sample is introduced into the capillary using hydrostatic sampling. The inlet of the capillary containing electrolyte is immersed in the sample and the height of the sample raised 10 cm for 30 s where low nanolitre volumes are siphoned into the capillary. After sample loading, the capillary is immediately immersed back into the electrolyte. The voltage is applied initiating the separation process.

4.3 Anion detection is based upon the principles of indirect UV detection. The UV-absorbing electrolyte anion is displaced charge-for-charge by the separated analyte anion. The analyte anion zone has a net decrease in background absorbance. This decrease in UV absorbance in quantitatively proportional to analyte anion concentration (see Fig. 9). Detector output polarity is reversed to provide positive mV response to the data system, and to make the negative absorbance peaks appear positive.

4.4 The analysis is complete once the last anion of interest is detected. The capillary is vacuum purged automatically by the system of any remaining sample and replenished with fresh electrolyte. The system now is ready for the next analysis.

5. Significance and Use

5.1 Capillary ion electrophoresis provides a simultaneous separation and determination of several inorganic anions using nanolitres of sample in a single injection. All anions present in the sample matrix will be visualized yielding an anionic profile of the sample.

5.2 Analysis time is less than 5 minutes with sufficient sensitivity for drinking water and wastewater applications. Time between samplings is less than seven minutes allowing for high sample throughput.

5.3 Minimal sample preparation is necessary for drinking water and wastewater matrices. Typically, only a dilution with water is needed.

5.4 This test method is intended as an alternative to other multi-analyte methods and various wet chemistries for the determination of inorganic anions in water and wastewater. Compared to other multi-analyte methods the major benefits of CIE are speed of analysis, simplicity, and reduced reagent consumption and operating costs.

6. Interferences

6.1 Analyte identification, quantitation, and possible comigration occur when one anion is in significant excess to other anions in the sample matrix. For two adjacent peaks, reliable quantitation can be achieved when the concentration differential is less than 100:1. As the resolution between two anion peaks increase so does the tolerated concentration differential. In samples containing 1000 mg/L Cl, 1 mg/L SO_4 can be resolved and quantitated, however, the high Cl will interfere with Br and NO_2 quantitation.

6.2 Dissolved carbonate, detected as HCO_3^{-1}, is an anion present in all aqueous samples, especially alkaline samples. Carbonate concentrations greater than 500 mg/L will interfere with PO_4 quantitation.

6.3 Monovalent organic acids, except for formate, and neutral organics commonly found in wastewater migrate later in the electropherogram, after carbonate, and do not interfere. Formate, a common organic acid found in environmental samples, migrates shortly after fluoride but before phosphate. Formate concentrations greater than 5 mg/L will interfere with fluoride identification and quantitation. Inclusion of 2 mg/L formate into the mixed anion working solution aids in fluoride and formate identification and quantitation.

6.4 Divalent organic acids usually found in wastewater migrate after phosphate. At high concentrations, greater than 10 mg/L, they may interfere with phosphate identification and quantitation.

6.5 Chlorate also migrates after phosphate and at concentrations greater than 10 mg/L will interfere with phosphate identification and quantitation. Inclusion of 5 mg/L chlorate into the mixed anion working solution aids in phosphate and chlorate identification and quantitation.

6.6 As analyte concentration increases, analyte peak shape becomes asymmetrical. If adjacent analyte peaks are not baseline resolved, the data system will drop a perpendicular between them to the baseline. This causes a decrease in peak area for both analyte peaks and a low bias for analyte amounts. For optimal quantitation, insure that adjacent peaks are fully resolved, if they are not, dilute the sample 1:1 with water.

7. Apparatus

7.1 *Capillary Ion Electrophoresis System*—the system consists of the following components, as shown in Fig. 10 or equivalent:[4]

7.1.1 *High Voltage Power Supply*, capable of generating voltage (potential) between 0 and minus 30 kV relative to ground with the capability working in a constant current mode.

7.1.2 *Covered Sample Carousel*, to prevent environmental contamination of the samples and electrolytes during a multi-sample batch analysis.

7.1.3 *Sample Introduction Mechanism*, capable of hydrostatic sampling technique, using gravity, positive pressure, or equivalent.

7.1.4 *Capillary Purge Mechanism*, to purge the capillary after every analysis with fresh electrolyte to eliminate any interference from the previous sample matrix, and to clean the capillary with other reagent, such as sodium hydroxide.

7.1.5 *UV Detector*, having the capability of monitoring 254 nm, or equivalent, with a time constant of 0.3 s.

7.1.6 *Fused Silica Capillary*—A 75 µm (inner diameter) x 375 µm (outer diameter) x 60 cm (length) having a polymer coating for flexibility, and noncoated section to act as the cell window for UV detection.[4]

7.1.7 *Constant Temperature Compartment*—To keep the samples, capillary, and electrolytes at constant temperature.

7.2 *Data System*—A computer system that can acquire data at 20 points/s minimum, express migration time in minutes to three decimal places, use midpoint of the analyte peak width, or center of gravity, to determine the analyte migration time, use normalized migration times with respect to a reference peak for qualitative identification, use time corrected peak area response for analyte quantitation, and express results in concentration units.[4]

NOTE 2—It is recommended that integrators or standard chromatographic data processing not be used with this test method.

7.3 *Anion Exchange Cartridges in the Hydroxide Form.*[5]

7.4 *Plastic Syringe*, 20-mL, disposable.

7.5 *Vacuum Filtration Apparatus*, capable for filtering 100 mL of reagent through a 0.45 µm aqueous filter.

8. Reagents and Materials

8.1 *Purity of Reagents*—Unless otherwise indicated, it is intended that all reagents shall conform to the reagent grade specification of the Analytical Reagents of the American Chemical Society, where such specifications are available.[6] Other grades may be used, provided it is first ascertained that the reagent is of sufficient high purity to permit its use without lessening the performance or accuracy of the determination. Reagent chemicals shall be used for all tests.

NOTE 3—Calibration and detection limits of this test method are biased by the purity of the reagents.

8.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean Type I reagent water conforming or exceeding specification D 1193. Freshly drawn water should be used for preparation of all stock and working standards, electrolytes, and solutions.[7] Performance and detection limits of this test method are limited by the purity of reagent water, especially TOC.

8.3 *Reagent Blank*—Reagent water, or any other solution, used to preserve or dilute the sample.

8.4 *Individual Anion Solution, Stock*

NOTE 4—It is suggested that certified individual 1000 mg/L anion standards be purchased for use with this test method.

NOTE 5—All weights given are for anhydrous or dried salts. Reagent purity must be accounted for in order to calculate true value concentration. Certify against NIST traceable standards.

8.4.1 *Bromide Solution, Standard (1.0 mL = 1.00 mg Bromide)*—Dry approximately 0.5 g of sodium bromide (NaBr) for 6 h at 150°C and cool in a desiccator. Dissolve 0.128 g of the dry salt in a 100 mL volumetric flask with water, and fill to mark with water.

8.4.2 *Chloride Solution, Standard (1.0 mL = 1.00 mg Chloride)*—Dry approximately 0.5 g of sodium chloride (NaCl) for 1 h at 100°C and cool in a desiccator. Dissolve 0.165 g of the dry salt in a 100 mL a volumetric flask with water, and fill to mark with water.

8.4.3 *Fluoride Solution, Standard (1.0 mL = 1.00 mg Fluoride)*—Dry approximately 0.5 g of sodium fluoride (NaF) for 1 h at 100°C and cool in a desiccator. Dissolve 0.221 g of the dry salt in a 100 mL volumetric flask with water, and fill to mark with water.

8.4.4 *Formate Solution, Standard (1.0 mL = 1.00 mg Formate)*—Dissolve 0.151 g of sodium formate in a 100-mL volumetric flask with water, and fill to mark with water.

8.4.5 *Nitrate Solution, Standard (1.0 mL = 1.00 mg Nitrate)*—Dry approximately 0.5 g of sodium nitrate ($NaNO_3$) for 48 h at 105°C and cool in a desiccator. Dissolve 0.137 g of the dry salt in a 100-mL volumetric flask with water, and fill to mark with water.

8.4.6 *Nitrite Solution, Standard (1.0 mL = 1.00 mg Nitrite)*—Dry approximately 0.5 g of sodium nitrite ($NaNO_2$) for 24 h in a desiccator containing concentrated sulfuric acid.

[4] Available from Waters, 34 Maple St., Milford, MA 01757.

[5] Available from Alltech Associates, P/N 30254, 2051 Waukegan Rd., Deerfield, IL, 60015.

[6] *Reagent Chemicals, American Chemical Society Specifications*, Am. Chem. Soc., 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. Pharmacopoeia Convention, Inc. (USPC), Rockville, Md.

[7] Although the reagent water may exceed Specification D 1193, the reagent water needs to be periodically tested for bacterial contamination. Bacteria and their waste products may adversely affect system performance. As a guide, ASTM Type IA water specifies a total bacteria count of 10 colonies/L. Refer to Test Method F 488 for analysis procedure.

Dissolve 0.150 g of the dry salt in a 100-mL volumetric flask with water, and fill to mark with water. Store in a sterilized glass bottle. Refrigerate and prepare monthly.

NOTE 6—Nitrite is easily oxidized, especially in the presence of moisture. Use only fresh reagent.

NOTE 7—Prepare sterile bottles for storing nitrite solutions by heating for 1 h at 170°C in an air oven.

8.4.7 *Ortho-Phosphate Solution, Standard (1.0 mL = 1.00 mg o-Phosphate)*—Dissolve 0.150 g of anhydrous dibasic sodium phosphate (Na_2HPO_4) in a 100-mL volumetric flask with water, and fill to mark with water.

8.4.8 *Sulfate Solution, Standard (1.0 mL = 1.00 mg Sulfate)*—Dry approximately 0.5 g of anhydrous sodium sulfate (Na_2SO_4) for 1 h at 110°C and cool in a dessicator. Dissolve 0.148 g of the dry salt in a 100-mL volumetric flask with water, and fill to mark with water.

8.5 *Mixed Anion Solution, Working*—Prepare at least three different working standard concentrations for the analyte anions of interest bracketing the desired range of analysis, typically between 0.1 and 50 mg/L, and add 2 mg/L formate to all standards. Add an appropriate aliquot of Individual anion stock solution (see 8.4) to a prerinsed 100-mL volumetric flask, and dilute to 100 mL with water.

NOTE 8—Use 100 µL of Individual anion stock solution (see 8.4) per 100 mL for 1 mg/L anion.

NOTE 9—Anions of no interest may be omitted.

NOTE 10—The midrange mixed anion solution, working may be used for the determination of migration times and resolution described in 12.1.

8.6 *Calibration Verification Solution (CVS)*—A solution formulated by the laboratory of mixed analytes of known concentration prepared in water. The CVS solution must be prepared from a different source to the calibration standards.

8.7 *Performance Evaluation Solution (PES)*—A solution formulated by an independent source of mixed analytes of known concentration prepared in water. Ideally, the PES solution should be purchased from an independent source.

8.8 *Quality Control Solution (QCS)*—A solution of known analyte concentrations added to a synthetic sample matrix such as substitute wastewater that sufficiently challenges the test method.

8.9 *Buffer Solution (100 mM CHES/1 mM Calcium Gluconate)*—Dissolve 20.73 g of CHES (2-[N-Cyclohexylamino]-Ethane Sulfonic Acid) and 0.43 g of calcium gluconate in a 1-L volumetric flask with water, and dilute to 1 L with water. This concentrate may be stored in a capped glass or plastic container for up to one year.

8.10 *Chromate Concentrate Solution (100 mM Sodium Chromate)*—Dissolve 23.41 g of sodium chromate tetrahydrate ($Na_2CrO_4 \cdot 4\ H_2O$) in a 1-L volumetric flask with water, and dilute to 1 L with water. This concentrate may be stored in a capped glass or plastic container for up to one year.

8.11 *OFM Concentrate Solution (100 mM Tetradecyltrimethyl Ammonium Bromide)*—Dissolve 33.65 g of Tetradecyltrimethyl Ammonium Bromide (TTABr) in a 1-L volumetric flask with water, and dilute to 1 L with water. Store this solution in a capped glass or plastic container for up to one year.

NOTE 11—TTABr needs to be converted to the hydroxide form (TTAOH) for use with this test method. TTAOH is commercially available as 100 mM TTAOH, which is an equivalent substitute.[8]

8.12 *Sodium Hydroxide Solution (500 mM Sodium Hydroxide)*—Dissolve 20 g of sodium hydroxide (NaOH) in a 1-L plastic volumetric flask with water, and dilute to 1 L with water.

8.13 *Electrolyte Solution, Working (4.7 mM Chromate/4 mM TTAOH/10 mM CHES/0.1 mM Calcium Gluconate)*[9]—Wash the anion exchange cartridge in the hydroxide form (see 7.3) using the 20-mL plastic syringe (see 7.4) with 10 mL of 500 mM NaOH (see 8.12) followed by 10 mL of water. Discard the washings. Slowly pass 4-mL of the 100 mM TTABr solution (see 8.11) through the cartridge into a 100-mL volumetric flask. Rinse the cartridge with 20 mL of water, adding the washing to the volumetric flask.

NOTE 12—The above procedure is used to convert the TTABr to TTAOH, which is used in the electrolyte. If using commercially available 100 mM TTAOH, the above conversion step is not necessary; substitute 4 mL of 100 mM TTAOH and continue below.

8.13.1 Into the 100-mL volumetric flask add 4.7 mL of chromate concentrate solution (see 8.10) and 10 mL of buffer solution (see 8.9). Mix and dilute to 100 mL with water. The natural pH of the electrolyte should be 9 ± 0.1. Filter and degas using the vacuum filtration apparatus. Store the any remaining electrolyte in a capped glass or plastic container at ambient temperature. The electrolyte is stable for one year.

9. Precautions

9.1 Chemicals used in this test method are typical of many useful laboratory chemicals, reagents, and cleaning solutions, which can be hazardous if not handled properly. Refer to Guide D 3856.

9.2 It is the responsibility of the user to prepare, handle, and dispose of chemical solutions in accordance with all applicable federal, state, and local regulations.

NOTE 13—**Warning:** This capillary electrophoresis method uses high voltage as a means for separating the analyte anions, and can be hazardous if not used properly. Use only those instruments that have all proper safety features.

10. Sampling

10.1 Collect samples in accordance with Practice D 3370.

10.2 Rinse sample containers with sample and discard to eliminate any contamination from the container. Fill to overflowing and cap to exclude air.

10.3 Analyze samples, as soon as possible, after collection. For nitrite, nitrate, and phosphate refrigerate the sample at 4°C after collection. Warm to room temperature before dilution and analysis.

10.4 At the laboratory, filter samples containing suspended solids through a prerinsed 0.45 µm aqueous compatible membrane filter before analysis.

[8] Available from Waters Corp., as IonSelect 100 mM OFM Hydroxide Concentrate, 100 mM TTAOH, P/N 49387.

[9] Available from Waters Corp. as IonSelect High MobilityAnion Electrolyte, P/N 49385.

10.5 If sample dilution is required to remain within the scope of this test method, dilute with water only.

11. Preparation of Apparatus

11.1 Set up the CE and data system according to the manufacturer's instructions.

11.2 Program the CE system to maintain a constant temperature of 25 ± 0.5°C, or 5°C above ambient laboratory temperature. Fill the electrolyte reservoirs with fresh chromate electrolyte working solution (see 8.13), and allow 10 minutes for thermal equilibration.

11.3 Condition a new capability (see 7.1.6) with 500 mM NaOH solution (see 8.12) for 5 minutes followed by water for 5 minutes. Purge the capillary with electrolyte (see 8.13) for 3 minutes.

11.4 Apply 15 kV of voltage and test for current. The current should be 14 ± 1 µA. If no current is observed, then there is a bubble, or blockage, or both, in the capillary. Degas the chromate electrolyte working solution and retry. If still no current, replace the capillary.

11.5 Set the UV detector to 254 nm detection, or equivalent. Zero the detector to 0.000 absorbance. UV offset is less than 0.1 AU.

11.6 Program the CE system for constant current of 14 µA.

11.7 Program the CE system for a hydrostatic sampling of 30 s. Approximately 37 nL of sample is siphoned into the capillary. Different sampling times may be used provided that the samples and standards are analyzed identically.

11.8 Program the CE system for 1 minute purge with the chromate electrolyte working solution between each analysis. Using a 15 psi vacuum purge mechanism, one 60-cm capillary volume can be displaced in 30 s.

11.9 Program the data system for an acquisition rate of at least 20 points/s. Program the data system to identify analyte peaks based upon normalized migration time using Cl as the reference peak, and to quantitate analyte peak response using time corrected peak area.

NOTE 14—Under the analysis conditions Cl is always the first peak in the electropherogram, and can be used as migration time reference peak.

12. Calibration

12.1 *Determination of Migration Times (Calibrate Daily)*—The migration time of an anion is dependent upon the electrolyte composition, pH, capillary surface and length, applied voltage, the ionic strength of the sample, and temperature. For every fresh electrolyte determine the analyte migration time, in min to the third decimal place, of the midrange mixed anion standard working solution (see 8.5), described in Section 11. Use the midpoint of analyte peak width as the determinant of analyte migration time.

NOTE 15—Analyte peak apex may be used as the migration time determinant, but potential analyte misidentification may result with asymmetrical peak shape at high analyte concentrations.

12.2 Analyze the blank (see 8.3) and at least three working mg/L solutions (see 8.5), using the set-up described in Section 11. For each anion concentration (X-axis) plot time corrected peak area response (Y-axis). Determine the best linear calibration line through the data points, or use the linear regression calibration routine (linear through zero) available in the data system.

NOTE 16—Do not use peak height for calibration. Peak area is directly related to migration time, that is, for the same analyte concentration, increasing migration time give increasing peak area.

12.2.1 The r^2 (coefficient of determination) values should be greater than 0.995; typical r^2 values obtained from the interlaboratory collaborative are given in Table A2.1.

12.3 Calibrate daily and with each change in electrolyte, and validate by analyzing the CVS solution (see 8.6) according to procedure in 16.4.

12.4 After validation of linear multiple point calibration, a single point calibration solution can be used between 0.1 and 50 mg/L for recalibration provided the quality control requirements in 16.4 are met.

13. Procedure

13.1 Dilute the sample, if necessary with water, to remain within the scope (see 1.2 and 1.3) and calibration of this test method. Refer to A1.5.1.

13.2 Analyze all blanks (see 8.3), standards (see 8.5), and samples as described in Section 11 using the quality control criteria described in 16.5-16.9. Refer to Figs. 1-6 for representative anion standard, detection limit standard, substitute wastewater, drinking water, and wastewater electropherograms.

13.3 Analyze all blanks, calibration standards, samples, and quality control solutions in singlicate.

13.3.1 *Optional*—Duplicate analyses are preferred due to short analysis times.

NOTE 17—Collaborative data was acquired, submitted and evaluated as the average of duplicate samplings.

13.4 After 20 sample analyses, or batch, analyze the QCS solution (see 8.8) If necessary, recalibrate using a single mixed anion standard working solution (see 8.5), and replace analyte migration time.

NOTE 18—A change in analyte migration time of the mixed anion standard working solution by more than +5% suggests that components in the previously analyzed sample matrices have contaminated the capillary surface. Continue but wash the capillary with NaOH solution (see 8.12) before the next change in electrolyte.

14. Calculation

14.1 Relate the time corrected peak area response for each analyte with the calibration curve generated in 12.2 to determine mg/L concentration of analyte anion. If the sample was diluted prior to analysis, then multiply mg/L anion by the dilution factor to obtain the original sample concentration, as follows:

$$\text{Original Sample mg/L Analyte} = (A \, x \, SF) \quad (1)$$

where:

A = analyte concentration determined from the calibration curve, in mg/L, and
SF = scale or dilution factor.

15. Report Format

15.1 The sample analysis report should contain the sample name, analyte anion name, migration time reported to three decimal places, migration time ratio, peak area, time corrected peak area, sample dilution, and original solution analyte concentration.

15.1.1 *Optional*—Report analysis method parameters, date of sample data acquisition, and date of result processing for documentation and validation purposes.

16. Quality Control

16.1 Before this test method is applied to the analysis of unknown samples, the analyst should establish control according to procedures recommended in Practice D 5847, and Guide D 5810.

16.2 The laboratory using this test should perform an initial demonstration of laboratory capability according to procedures outlines in Practice D 5847.

NOTE 19—Certified performance evaluation solutions (PES) and QC solutions (QCS and CVS) are commercially available and recommended.

16.3 *Initial Demonstration of Performance*—Analyze seven replicates of a performance evaluation solution (PES) (see 8.7). The analyte concentration mean and standard deviation of the seven replicates should be calculated and compared to the test methods single operator precision for equivalent concentrations in reagent water given in Section 17.

16.3.1 Repeat the seven replicate analysis protocol before using a freshly prepared QVS solution (see 8.6) and QCS solution (see 8.8) for the first time. Calculate the standard deviation and compare with previous results using the student *t*-test. If no significant difference is noted, then use the combined standard deviation to determine the QC limits, generally the mean ± three standard deviations, for the QCV and QCS solutions.

16.4 *Calibration Verification*—After calibration, verify the calibration linearity and acceptable instrument performance using a calibration verification solution (see 8.6) treated as an unknown. If the determined CVS concentrations (see 8.6) are not within ± 3 standard deviations of the known true values as described in 16.3.1, the calibration solutions may be out of control. Reanalyze, and if analyte concentration still falls outside the acceptable limits, fresh calibration solutions (see 8.5) are required. Successful CVS analyte concentration must be confirmed after recalibration before continuing with the test method.

16.5 Analyze a reagent blank (see 8.3) with each batch to check for contamination introduced by the laboratory or use of the test method.

16.6 *Quality Control Solution*—Analyze one QCS (see 8.8) after 20 samples, or batch. The analyte concentrations for the QCS should fall within ± 3 standard deviations of historical values for the equivalent concentration and matrix. They are determined as described in 16.3.1.

Upper Control Limit = Analyte Mean Value + 3 times the Standard Deviation
Lower Control Limit = Analyte Mean Value – 3 times the Standard Deviation

16.7 *Matrix Spike Recovery*—One matrix spike (MS) should be analyzed with each batch of samples to test method recovery. Spike a portion of one sample from each batch with a known concentration of analyte, prepared in accordance with Guide D 3856. The % recovery of the spike should fall within %recovery ± analyst %RSD for an equivalent spike concentration and matrix given in Tables 1-7. If it does not, an interference may be present and the data for the set of similar samples matrices must be qualified with a warning that the data are suspect, or an alternate test method should be used. Refer to Guide D 5810.

16.7.1 If the known analyte concentration is between 15 and 50 mg/L, then spike the sample solution to increase analyte concentration by 50 %.

16.7.2 If the known analyte concentration is less than 15 mg/L, then spike the sample solution to increase analyte concentration by 100 %, but not less than 2 mg/L.

16.7.3 Calculate the percent recovery of the spike using the following formula:

$$\% \text{ Recovery} = 100\ [A\ (V_s + V) - B\ V_s]/C\ V \quad (2)$$

where:
A = Analyte concentration (mg/L) in spiked sample,
B = Analyte concentration (mg/L) in unspiked sample,
C = Concentration (mg/L) of analyte in spiking solution,
V_s = Volume (mL) of sample used, and
V = Volume (mL) added with spike,

Evaluate the performance according to Practice D 5847.

16.8 *Method Precision*—One unknown sample should be analyzed in triplicate with each batch to test method precision. Calculate the standard deviation and use the *F*-test to compare with the single operator precision give in Tables 1-7 for the equivalent analyte concentration and matrix type. Evaluate performance according to Practice D 5847.

16.9 The laboratory may perform additional quality control as desired or appropriate.

17. Precision and Bias

17.1 The precision and bias data presented in this test method meet the requirements of Practice D 2777, and are given in Tables 1-7.

17.2 This test method interlaboratory collaborative was performed by 11 laboratories using one operator each. Four Youden-Pair spike concentrations for the seven analytes anions yielding eight analyte concentration levels. Test data was submitted for eleven reagent waters, eleven substitute wastewaters, 15 drinking waters, and 13 wastewater sample matrices.

17.3 The precision, bias, and matrix recovery of this test method per anion analyte in four tested sample matrices are based upon the analyte true value, calculated using weight, volume, and purity. True value spiking solution concentrations are given in Table A1.4.

17.4 The bias and matrix recovery statements for less than 2 mg/L of chloride, sulfate, and nitrate in naturally occurring sample matrices may be misleading due to spiking of small analyte concentration into a high naturally occurring analyte concentration observed with the matrix blank. The commonly occurring analyte concentrations observed in the sample matrix blanks for the naturally occurring tested matrices are given in Table A1.5.

TABLE 1 Precision, Bias, and Matrix Recovery for Chloride

Matrix	No. of Values	True Value	Mean Result	Bias Versus True Value	Recovery Versus True Value	Interlab Std Dev *S(t)*	Interlab %RSD	Single Operator Std Dev. *S(o)*	Analyst %RSD
Reagent water	9	0.50	0.55	0.05	110.0	0.11	19.8		
	10	0.71	0.69	–0.02	97.2	0.08	11.5	0.05	7.5
	10	2.00	1.97	–0.03	98.5	0.14	6.8		
	9	2.98	2.97	–0.01	99.7	0.11	3.8	0.05	2.1
	10	14.92	14.76	–0.16	98.9	0.61	4.2		
	10	19.91	19.81	–0.10	99.5	0.81	4.1	0.48	2.8
	10	39.81	38.58	–1.23	96.9	1.43	3.7		
	10	49.76	48.70	–1.06	97.9	1.94	4.0	1.36	3.1
Substitute wastewater	9	0.50	0.46	–0.04	92.0	0.51	111.1		
	9	0.71	0.43	–0.28	60.6	0.69	160.7	0.42	93.8
	9	2.00	1.52	–0.48	76.0	0.68	45.0		
	9	2.98	2.58	–0.40	86.6	0.63	24.5	0.50	24.3
	9	14.92	14.29	–0.63	95.8	1.02	7.1		
	9	19.91	18.93	–0.98	95.1	1.24	6.6	0.60	3.6
	9	39.81	37.34	–2.47	93.8	5.44	14.6		
	9	49.76	47.54	–2.22	95.5	3.13	6.6	4.43	10.4
Drinking water	12	0.50	0.63	0.13	126.0	0.67	106.1		
	12	0.71	0.75	0.04	105.6	0.34	45.5	0.40	57.2
	12	2.00	2.15	0.15	107.5	0.51	23.6		
	12	2.98	2.95	–0.03	99.0	0.39	13.1	0.47	18.5
	12	14.92	14.54	–0.38	97.5	0.71	4.9		
	12	19.91	19.09	–0.82	95.9	1.11	5.8	0.37	2.2
	12	39.81	38.38	–1.43	96.4	1.56	4.1		
	12	49.76	47.97	–1.79	96.4	2.19	4.6	1.26	3.9
″Real″ Wastewater	9	0.50	0.42	–0.08	84.0	0.34	81.0		
	10	0.71	0.47	–0.24	66.2	0.34	72.6	0.26	59.3
	10	2.00	1.56	–0.44	78.0	0.51	32.7		
	9	2.98	2.78	–0.20	93.3	0.19	6.8	0.37	17.3
	10	14.92	14.29	–0.63	95.8	0.63	4.4		
	10	19.91	18.83	–1.08	94.6	0.78	4.1	0.46	2.8
	9	39.81	37.01	–2.80	93.0	2.78	7.5		
	10	49.76	48.24	–1.52	96.9	3.15	6.5	2.54	6.0

17.5 The high nitrate bias and %recovery noted for the 0.5 mg/L NO_3 spike solution are attributed to the spiking solution containing 50 mg/L nitrite and 0.5 mg/L nitrate. Refer to Annex Table A1.4, Solution 3. Some of the nitrite converted to nitrate prior to analysis. Similar NO_x conversion effect is observed with the 2-mg/L nitrate and 2 mg/L nitrite spike, Solution 7.

17.6 All collaborative participants used the premade chromate electrolyte.[9] Ten laboratories used a Waters CIA Analyzer with Millennium Data Processing Software, and one laboratory used a Agilent CE System with Diode Array Detector that provided equivalent results.

NOTE 20—Refer to reference B1.16 and Agilent (the former HP company) website for recommended operating conditions.

18. Keywords

18.1 anion; capillary electrophoresis; drinking water; ion analysis; reagent water; substitute wastewater; wastewater

TABLE 2 Precision, Bias, and Matrix Recovery for Bromide

Matrix	No. of Values	True Value	Mean Result	Bias Versus True Value	Recovery Versus True Value	Interlab Std Dev *S(t)*	Interlab %RSD	Single Operator Std Dev. *S(o)*	Analyst %RSD
Reagent water	10	0.51	0.60	0.09	117.6	0.19	31.0		
	10	0.70	0.83	0.13	118.6	0.23	28.2	0.10	14.6
	10	2.00	2.06	0.06	103.0	0.14	6.6		
	10	3.01	2.88	–0.13	95.7	0.23	7.9	0.15	6.3
	10	14.93	15.00	0.07	100.5	0.58	3.9		
	10	19.91	19.32	–0.59	97.0	0.97	5.0	0.75	4.4
	10	39.81	39.66	–0.15	99.6	1.24	3.1		
	10	49.77	50.04	0.27	100.5	2.94	5.9	1.61	3.6
Substitute wastewater	9	0.51	0.67	0.16	131.4	0.19	28.8		
	9	0.70	0.96	0.26	137.1	0.21	21.8	0.08	9.3
	9	2.00	2.14	0.14	107.0	0.22	10.2		
	9	3.01	2.72	–0.29	90.4	0.35	12.8	0.17	7.0
	9	14.93	14.70	–0.23	98.5	0.58	3.9		
	9	19.91	18.91	–1.00	95.0	2.62	13.8	1.63	9.7
	9	39.81	38.76	–1.05	97.4	1.11	2.9		
	9	49.77	48.81	–0.96	98.1	1.52	3.1	0.48	1.1
Drinking water	13	0.51	0.58	0.07	113.7	0.25	43.4		
	13	0.70	0.83	0.13	118.6	0.22	26.5	0.14	19.9
	13	2.00	1.98	–0.02	99.0	0.25	12.5		
	13	3.01	2.56	–0.45	85.0	0.25	9.7	0.15	6.8
	13	14.93	14.63	–0.30	98.0	0.50	3.4		
	13	19.91	19.22	–0.69	96.5	1.10	5.7	0.77	4.6
	13	39.81	38.97	–0.84	97.9	1.99	5.1		
	13	49.77	48.74	–1.03	97.9	1.49	3.1	1.13	2.6
″Real″ Wastewater	11	0.51	0.59	0.08	115.7	0.11	19.3		
	12	0.70	0.78	0.08	111.4	0.19	24.4	0.10	14.0
	11	2.00	2.08	0.08	104.0	0.13	6.3		
	12	3.01	2.70	–0.31	89.7	0.41	15.1	0.27	11.5
	12	14.93	15.16	0.23	101.5	0.90	6.0		
	11	19.91	19.46	–0.45	97.7	1.63	8.4	1.09	6.3
	12	39.81	40.24	0.43	101.1	2.27	5.7		
	12	49.77	49.97	0.20	100.4	2.52	5.0	0.91	2.0

TABLE 3 Precision, Bias, and Matrix Recovery for Nitrite

Matrix	No. of Values	True Value	Mean Result	Bias Versus True Value	Recovery Versus True Value	Interlab Std Dev *S(t)*	Interlab %RSD	Single Operator Std Dev. *S(o)*	Analyst %RSD
Reagent water	9	0.50	0.62	0.12	124.0	0.16	26.1		
	9	0.70	0.72	0.02	102.9	0.08	10.5	0.05	7.1
	10	2.00	1.31	–0.69	65.5	0.25	19.2		
	10	2.98	3.11	0.13	104.4	0.17	5.4	0.13	6.0
	10	14.86	14.70	–0.16	98.9	0.47	3.2		
	10	19.81	19.88	0.07	100.4	0.70	3.5	0.27	1.5
	10	39.61	39.90	0.29	100.7	0.88	2.2		
	10	49.52	48.24	–1.28	97.4	1.34	2.8	1.25	2.8
Substitute wastewater	9	0.50	0.37	–0.13	74.0	0.22	59.7		
	9	0.70	0.59	–0.11	84.3	0.28	48.1	0.21	43.2
	10	2.00	1.25	–0.75	62.5	0.38	30.8		
	9	2.98	2.62	–0.36	87.9	0.82	31.4	0.43	22.1
	9	14.86	14.40	–0.46	96.9	0.58	4.0		
	10	19.81	19.50	–0.31	98.4	1.66	8.5	0.81	4.8
	10	39.61	39.97	0.36	100.9	2.02	5.0		
	9	49.52	49.09	–0.43	99.1	3.03	6.2	2.11	4.7
Drinking water	11	0.50	0.52	0.02	104.0	0.08	14.4		
	12	0.70	0.74	0.04	105.7	0.17	23.3	0.09	13.5
	12	2.00	1.30	–0.70	65.0	0.21	15.9		
	12	2.98	2.97	–0.01	99.7	0.14	4.6	0.16	7.4
	11	14.86	14.60	–0.26	98.3	0.40	2.8		
	11	19.81	19.82	0.01	100.1	0.59	3.0	0.26	1.5
	11	39.61	39.35	–0.26	99.3	0.99	2.5		
	12	49.52	49.14	–0.38	99.2	1.93	3.9	0.64	1.5
″Real″ Wastewater	9	0.50	0.55	0.05	110.0	0.13	24.5		
	10	0.70	0.73	0.03	104.3	0.24	32.9	0.07	10.8
	9	2.00	1.27	–0.73	63.5	0.18	14.2		
	10	2.98	2.99	0.01	100.3	0.19	6.2	0.15	7.0
	10	14.86	14.55	–0.31	97.9	0.46	3.1		
	10	19.81	19.68	–0.13	99.3	0.71	3.6	0.38	2.2
	9	39.61	39.21	–0.40	99.0	1.03	2.6		
	9	49.52	47.27	–2.25	95.5	3.50	7.4	2.40	5.6

TABLE 4 Precision, Bias, and Matrix Recovery for Sulfate

Matrix	No. of Values	True Value	Mean Result	Bias Versus True Value	Recovery Versus True Value	Interlab Std Dev *S(t)*	Interlab %RSD	Single Operator Std Dev. *S(o)*	Analyst %RSD
Reagent water	9	0.49	0.49	0.00	100.0	0.18	37.5		
	10	0.70	0.71	0.01	101.4	0.20	29.2	0.05	8.3
	10	1.98	2.04	0.06	103.0	0.19	9.7		
	10	2.98	3.09	0.11	103.7	0.24	7.9	0.06	2.5
	10	14.86	14.67	–0.19	98.7	0.57	4.0		
	10	19.81	19.67	–0.14	99.3	0.73	3.8	0.44	2.6
	10	39.60	39.66	0.06	100.2	0.92	2.4		
	10	49.51	49.27	–0.24	99.5	1.26	2.6	0.49	1.1
Substitute wastewater	9	0.49	0.38	–0.11	77.6	0.25	66.9		
	9	0.70	0.51	–0.19	72.9	0.08	16.4	0.18	39.3
	9	1.98	1.83	–0.15	92.4	0.29	16.2		
	9	2.98	2.86	–0.12	96.0	0.31	11.2	0.20	8.6
	9	14.86	14.19	–0.67	95.5	1.06	7.7		
	9	19.81	19.23	–0.58	97.1	0.97	5.2	0.46	2.8
	9	39.60	38.45	–1.15	97.1	1.33	3.6		
	9	49.51	47.75	–1.76	96.4	1.43	3.1	0.75	1.8
Drinking water	12	0.49	0.41	–0.08	83.7	0.21	52.8		
	12	0.70	0.41	–0.29	58.6	0.20	50.3	0.14	34.3
	13	1.98	1.77	–0.21	89.4	0.53	30.3		
	13	2.98	2.68	–0.30	89.9	0.42	16.2	0.27	12.1
	13	14.86	14.25	–0.61	95.9	1.11	8.0		
	12	19.81	19.31	–0.50	97.5	1.39	7.4	1.48	8.9
	12	39.60	38.58	–1.02	97.4	1.96	5.2		
	13	49.51	48.43	–1.08	97.8	2.04	4.3	1.44	3.3
″Real″ Wastewater	10	0.49	0.37	–0.12	75.5	0.39	106.4		
	11	0.70	0.16	–0.54	22.9	1.19	765.2	0.47	179.6
	11	1.98	1.57	–0.41	79.3	0.87	55.4		
	11	2.98	2.53	–0.45	84.9	0.64	25.4	0.24	11.9
	11	14.86	14.69	–0.17	98.9	1.26	8.6		
	10	19.81	19.38	–0.43	97.8	0.90	4.6	0.57	3.4
	11	39.60	38.74	–0.86	97.8	1.71	4.4		
	10	49.51	48.36	–1.15	97.7	1.51	3.1	0.47	1.1

TABLE 5 Precision, Bias, and Matrix Recovery for Nitrate

Matrix	No. of Values	True Value	Mean Result	Bias Versus True Value	Recovery Versus True Value	Interlab Std Dev *S(t)*	Interlab %RSD	Single Operator Std Dev. *S(o)*	Analyst %RSD
Reagent water	10	0.50	1.02	0.52	204.00	0.08	7.4		
	10	0.69	0.71	0.02	102.90	0.08	11.6	0.06	6.4
	11	1.99	2.83	0.84	142.21	0.23	8.1		
	11	2.97	2.89	–0.08	97.31	0.18	6.4	0.14	5.0
	11	14.91	14.77	–0.14	99.06	0.44	3.0		
	11	19.18	19.77	0.59	103.08	0.64	3.2	0.24	1.4
	10	39.86	39.09	–0.77	98.07	1.43	3.7		
	10	49.77	48.93	–0.84	98.31	1.72	3.5	0.62	1.4
Substitute wastewater	11	0.50	1.18	0.68	236.00	0.41	34.9		
	10	0.69	0.55	–0.14	79.71	0.30	55.3	0.42	4.9
	10	1.99	2.70	0.71	135.68	0.42	15.4		
	10	2.97	2.33	–0.64	78.45	1.10	47.3	0.39	15.4
	9	14.91	14.29	–0.62	95.84	0.78	5.4		
	10	19.18	18.69	–0.49	97.45	1.46	7.8	0.25	1.5
	11	39.86	37.70	–2.16	94.58	1.93	5.1		
	11	49.77	47.78	–1.99	96.00	2.18	4.6	1.62	3.8
Drinking water	11	0.50	1.06	0.56	212.00	0.19	18.1		
	11	0.69	0.65	–0.04	94.20	0.06	8.7	0.12	14.4
	12	1.99	3.05	1.06	153.27	0.39	12.8		
	11	2.97	3.01	0.04	101.35	0.22	7.2	0.33	10.8
	12	14.91	14.69	–0.22	98.52	0.62	4.2		
	12	19.18	20.05	0.87	104.54	0.88	4.4	0.46	2.7
	12	39.86	39.31	–0.55	98.62	1.67	4.3		
	12	49.77	48.93	–0.84	98.31	1.43	2.9	0.78	1.8
″Real″ Wastewater	11	0.50	0.94	0.44	188.00	0.80	84.7		
	10	0.69	0.69	0.00	100.00	0.09	13.3	0.39	47.6
	10	1.99	3.00	1.01	150.75	0.38	12.7		
	10	2.97	3.01	0.04	101.35	0.20	6.6	0.23	7.8
	11	14.91	14.52	–0.39	97.38	0.66	4.6		
	11	19.18	19.26	0.08	100.42	0.77	4.0	0.77	4.6
	11	39.86	39.13	–0.73	98.17	1.78	4.6		
	11	49.77	49.17	–0.60	98.79	2.26	4.6	0.93	2.1

TABLE 6 Precision, Bias, and Matrix Recovery for Fluoride

Matrix	No. of Values	True Value	Mean Result	Bias Versus True Value	Recovery Versus True Value	Interlab Std Dev *S(t)*	Interlab %RSD	Single Operator Std Dev. *S(o)*	Analyst %RSD
Reagent water	10	0.50	0.51	0.01	102.00	11.00	11.4		
	10	0.71	0.73	0.02	102.82	7.90	8.1	0.02	2.9
	10	2.00	2.05	0.05	102.50	3.60	3.7		
	10	3.00	2.96	–0.04	98.67	4.40	4.6	0.09	3.4
	10	6.99	7.02	0.03	100.43	5.40	5.6		
	10	9.99	9.79	–0.20	98.00	4.60	4.8	0.13	1.6
	10	19.98	19.60	–0.38	98.10	3.80	3.9		
	10	24.99	24.51	–0.48	98.08	4.80	4.9	0.74	3.4
Substitute wastewater	10	0.50	0.50	0.00	100.00	0.09	18.0		
	10	0.71	0.71	0.00	100.00	0.09	12.0	0.01	2.3
	10	2.00	1.98	–0.02	99.00	0.12	6.0		
	10	3.00	2.94	–0.06	98.00	0.10	3.4	0.06	2.6
	10	6.99	6.92	–0.07	99.00	0.28	4.1		
	9	9.99	9.94	–0.05	99.50	0.46	4.7	0.28	3.3
	10	19.98	19.67	–0.31	98.45	0.94	4.8		
	10	24.99	24.78	–0.21	99.16	1.09	4.4	0.63	2.8
Drinking water	13	0.50	0.48	–0.02	96.00	0.06	12.9		
	13	0.71	0.68	–0.03	95.77	0.06	9.5	0.02	3.4
	13	2.00	1.96	–0.04	98.00	0.08	3.9		
	13	3.00	2.90	–0.10	96.67	0.10	3.4	0.08	3.5
	13	6.99	6.91	–0.08	98.86	0.25	3.6		
	13	9.99	9.91	–0.08	99.20	0.37	3.7	0.18	2.2
	13	19.98	19.94	–0.04	99.80	0.68	3.4		
	12	24.99	24.27	–0.72	97.12	1.63	6.7	1.30	5.9
″Real″ Wastewater	11	0.50	0.47	–0.03	94.00	0.08	16.9		
	11	0.71	0.68	–0.03	95.77	0.08	11.7	0.04	7.6
	11	2.00	1.96	–0.04	98.00	0.12	6.3		
	11	3.00	2.93	–0.07	97.67	0.18	6.2	0.09	3.5
	11	6.99	6.85	–0.14	98.00	0.26	3.8		
	10	9.99	9.56	–0.43	95.70	0.73	7.7	0.44	5.3
	11	19.98	20.06	0.08	100.40	1.23	6.1		
	11	24.99	25.12	0.13	100.52	1.34	5.3	0.32	1.4

TABLE 7 Precision, Bias, and Matrix Recovery for o-Phosphate

Matrix	No. of Values	True Value	Mean Result	Bias Versus True Value	Recovery Versus True Value	Interlab Std Dev *S(t)*	Interlab %RSD	Single Operator Std Dev. *S(o)*	Analyst %RSD
Reagent water	10	0.50	0.41	–0.09	82.00	0.12	29.6		
	9	0.69	0.51	–0.18	73.91	0.13	26.6	0.03	7.2
	10	1.99	1.88	–0.11	94.47	0.16	8.3		
	10	2.98	2.76	–0.22	92.62	0.14	4.9	0.08	3.2
	10	14.86	14.93	0.07	100.47	0.64	4.3		
	9	19.80	19.76	–0.04	99.80	1.00	5.1	0.85	4.9
	10	39.60	39.79	0.19	100.48	1.38	3.5		
	10	49.51	50.10	0.59	101.19	1.76	3.5	0.72	1.6
Substitute wastewater	11	0.50	0.49	–0.01	98.00	0.15	30.0		
	10	0.69	0.59	–0.10	85.51	0.17	28.8	0.13	24.4
	11	1.99	1.92	–0.07	96.48	0.28	14.6		
	10	2.98	2.89	–0.09	96.98	0.22	7.6	0.18	7.5
	11	14.86	15.31	0.45	103.03	1.74	11.4		
	11	19.80	19.78	–0.02	99.90	1.16	5.9	0.84	4.8
	11	39.60	39.58	–0.02	99.95	2.72	6.9		
	11	49.51	49.19	–0.32	99.35	3.98	8.1	2.18	4.9
Drinking water	12	0.50	0.46	–0.04	92.00	0.14	30.0		
	13	0.69	0.55	–0.14	79.71	0.20	36.3	0.07	13.4
	13	1.99	1.89	–0.10	94.97	0.22	11.9		
	13	2.98	2.87	–0.11	96.31	0.24	8.5	0.07	2.8
	12	14.86	15.09	0.23	101.55	0.91	6.1		
	13	19.80	20.28	0.48	102.42	0.96	4.7	1.06	6.0
	13	39.60	40.37	0.77	101.94	2.15	5.3		
	13	49.51	50.75	1.24	102.50	3.14	6.2	1.03	2.3
″Real″ Wastewater	11	0.50	0.43	–0.07	86.00	0.17	39.1		
	11	0.69	0.53	–0.16	76.81	0.24	46.5	0.12	25.8
	11	1.99	1.72	–0.27	86.43	0.27	15.8		
	11	2.98	2.52	–0.46	84.56	0.48	19.2	0.30	14.0
	11	14.86	14.93	0.07	100.47	0.91	6.1		
	11	19.80	19.90	0.10	100.51	1.35	6.8	0.91	5.2
	11	39.60	38.98	–0.62	98.43	1.45	3.7		
	10	49.51	48.26	–1.25	97.48	1.80	3.7	0.82	1.9

ANNEX

(Mandatory Information)

A1. Data

A1.1 All data presented in the following tables conform and exceed the requirements of Practice D 2777–98. Data from eleven reagent waters, eleven substitute wastewater, 15 drinking water, and thirteen wastewater sample matrices, were tested using a set of four Youden-Pair concentrations for seven analyte anions. All submitted individual data points are the average of duplicate samplings.

A1.2 Calibration Linearity

A1.2.1 All laboratories used a provided set of four certified, mixed anion calibration solutions in concentrations between 0.5 mg/L and 50 mg/L, formulated in random concentrations given in Table A1.1. They were prepared from certified, individual 1000 mg/L stock standards.[10] No dilution was necessary.

TABLE A1.1 Collaborative Calibration Standard, mg/L Concentrations

Analyte Anion	Standard 1	Standard 2	Standard 3	Standard 4
Chloride	50	25	0.5	10
Bromide	0.5	25	10	50
Nitrite	25	0.5	50	10
Sulfate	10	25	0.5	50
Nitrate	25	0.5	50	10
Fluoride	5	0.5	10	25
Phosphate	50	25	0.5	10

A1.2.2 A linear through zero regression was used to calculate the calibration curve. The range coefficient of determination (r^2) values obtained from the collaborative is shown in Table A1.2.

A1.3 Quality Control Solution Preparation

A1.3.1 The quality control solution (QCS) also was used as the calibration verification solution (CVS).

[10] Obtained from APG Inc., Belpre, OH.

TABLE A1.2 Expected Range of (r^2) Coefficient of Determination

Anion / r^2	Average, n=29	Lowest	Highest
Chloride	0.99987	0.99959	0.99997
Bromide	0.99953	0.99878	0.99996
Nitrite	0.99983	0.99961	0.99999
Sulfate	0.99976	0.99901	0.99999
Nitrate	0.99957	0.99840	0.99999
Fluoride	0.99972	0.99797	0.99999
Phosphate	0.99982	0.99942	0.99999

TABLE A1.4 True Value Youden Pair Spiking mg/L Concentrations

Anion/TV	1	2	3	4	5	6	7	8	9
Chloride	0.71	2.00	2.98	14.92	39.81	19.91	49.76	0.50	10.20
Bromide	2.00	3.01	14.93	39.81	19.91	49.77	0.70	0.51	10.49
Nitrite	2.98	39.61	19.81	14.86	49.52	0.50	2.00	0.70	9.94
Sulfate	39.60	49.51	0.49	0.70	1.98	2.98	14.86	19.81	10.23
Nitrate	14.92	19.19	39.87	49.78	0.50	0.70	2.00	2.98	10.35
Fluoride	2.00	0.71	0.50	3.00	9.99	6.99	19.98	24.99	10.40
Phosphate	49.51	39.60	19.90	0.50	2.98	1.99	0.69	14.86	10.48

A1.3.2 The quality control solution (QCS) was manufactured and certified[10] as 100× concentrate, to replicate typical drinking water concentrations, and required 1:100 dilution with water before analysis. The QCS analyte concentrations, required control limits, and interlaboratory determined control limits based upon 82 analyses are given in Table A1.3.

A1.4 Youden Pair Spiking Solution Preparation

A1.4.1 Eight mixed anion, 100× concentrate, spiking solutions were prepared in accordance with the Reagents and Materials of the test method using anhydrous sodium salts. The mg/L concentrations of the eight standards followed the approved Youden Pair design: 0.5 and 0.7, 2 and 3, 15 and 20, 40 and 50 mg/L for all anions except fluoride, which is 0.5 and 0.7, 2 and 3, 7 and 10, 20 and 25 mg/L. The analyte true value concentrations were randomized among the eight spiking solutions as described in Table A1.4.

A1.4.2 A ninth solution containing approximately 10 mg/L of each analyte was used for method detection limit calculations.

A1.4.2.1 These solutions, kept at ambient temperature, were analyzed before and during the collaborative to monitor for accuracy and stability. The mg/L true value in was used to determine bias, matrix recovery, and the single operator and interlaboratory precision in the P and B tables in accordance with Practice D 2777.

A1.4.2.2 Solution 3 and 7 exhibited some conversion of nitrite to nitrate before analysis. This conversion is evident in the bias and % recovery for 0.5 mg/L and 2 mg/L nitrite and nitrate.

A1.5 Sample Matrix Preparation

A1.5.1 All participating laboratories provided and tested reagent water, substitute wastewater, naturally occurring drinking water, and naturally occurring wastewater. Before matrix spiking with the Youden Pair solutions, the sample matrix was evaluated, then appropriately diluted to give the highest anion concentration below 50 mg/L. The diluted sample matrix was used to dilute each Youden Pair spiking solution 1:100.

TABLE A1.3 Quality Control Acceptance Limits

Analyte Anion	True Value, mg/L	Certified Value, mg/L	Required 99 % Confidence Interval	Determined QCS Mean ± Std Dev, n = 82
Chloride	48.68	48.61 ± 0.12	43.99–52.96	47.64 ± 1.53
Bromide	0.00	0.00	0.00	0.00
Nitrite	2.87	2.90 ± 0.07	2.39–3.26	2.88 ± 0.19
Sulfate	35.69	35.63 ± 0.25	29.54–40.53	35.02 ± 1.21
Nitrate	15.76	15.78 ± 0.15	12.80–18.39	15.33 ± 4.35
Fluoride	1.69	1.68 ± 0.01	1.49–1.87	1.67 ± 0.09
Phosphate	5.47	5.55 ± 0.12	4.78–6.20	5.58 ± 0.28

A1.5.2 Reagent water was used as-is. Substitute wastewater was diluted 1:20 with water. Naturally occurring drinking water was used as-is or diluted 1:5 with water. Naturally occurring wastewater was diluted between 1:3 and 1:20, except one which required a 1:1000 dilution due to high chloride.

A1.5.3 Due to the anion content of the naturally occurring drinking water and "real" wastewater matrices, some of the reported spike matrix results exceeded the scope of this test method. Linearity and matrix recovery data obtained from the collaborative indicated that these data are acceptable, and extended the useful range of this test method.

A1.5.4 Due to the anion content of the naturally occurring sample matrices given in Table A1.5, the low concentration bias and recovery may be misleading because of spiking a low anion concentration increment into a large naturally occurring concentration of the same anion.

A1.6 Test Method Detection Limits

A1.6.1 Spiking Solution No. 9, containing 10 mg/L of each analyte, was diluted 1:50 with water and was used for detection limit calculations. Seven replicate samplings were run, and the mean and standard deviation were calculated. The mean time corrected peak area response was given the true value of the solution No. 9, and from a simple proportion, the standard deviation was calculated as mg/L.

$$\text{Std Dev, mg/L} = \frac{(\text{True Value Conc Sol'n No. 9, mg/L})(\text{Response Std Dev})}{\text{Ave Response of Sol'n No. 9}} \quad (A1.1)$$

A1.6.2 Method detection limits were derived using EPA protocol and the student t-test at 6 df, as follows:

$$\text{The method detection limit (MDL)} = (3.14)(\text{Std Dev, mg/L}) \quad (A1.2)$$

A1.6.3 The upper and lower confidence limits were calculated as;

95 % Confidence Interval: LCL (Lower Confidence Limit) - 0.64 × MDL
UCL (Upper Confidence Limit) = 2.20 × MDL

A1.6.4 Method detection limits are given in Table A1.6.

TABLE A1.5 Blank Analyte Concentrations for Naturally Occurring Sample Matrices

Data in mg/L	Chloride	Sulfate	Nitrate
Drinking water	0.7 to 41.9	0.5 to 33.6	0.2 to 6.5
Substitute wastewater	20.5 to 25.5	3.2 to 4.0	Not Detected
"Real" wastewater	0.9 to 43.4	0.5 to 50.4	0.3 to 23.0

TABLE A1.6 Method Detection Limits

Anion	mg/L Solution Concentration	Method Detection MDL, mg/L	95 % Confidence Interval mg/L
Chloride	0.204	0.073	0.047 to 0.161
Bromide	0.210	0.132	0.084 to 0.290
Nitrite	0.199	0.102	0.065 to 0.223
Sulfate	0.205	0.066	0.042 to 0.145
Nitrate	0.207	0.082	0.052 to 0.180
Fluoride	0.208	0.032	0.020 to 0.070
Phosphate	0.210	0.102	0.065 to 0.224

APPENDIX

(Nonmandatory Information)

X1. SUGGESTED BACKGROUND REFERENCES

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