4140

Inorganic Anions by Capillary Ion Electrophoresis

Approved by Standard Methods Committee, 2004. Editorial revisions, 2011 and 2020. Joint Task Group: 20th Edition—Roy-Keith Smith (chair), James Krol, Yuefeng Xie.

4140 A. Introduction

Determination of common inorganic anions such as fluoride, chloride, bromide, nitrite, nitrate, orthophosphate, and sulfate is a significant component of water quality analysis. Instrumental techniques that can determine multiple analytes in a single analysis [i.e., ion chromatography (Section 4110) and capillary ion electrophoresis] offer significant time and operating cost savings over traditional single-analyte wet chemical analysis.

Capillary ion electrophoresis is rapid (complete analysis in less than 5 min) and provides additional anion information (i.e., organic acids) not available with isocratic ion chromatography (IC). Operating costs are significantly less than those of ion chromatography. Capillary ion electrophoresis can detect all anions present in the sample matrix, providing an anionic fingerprint.

Anion selectivity of capillary ion electrophoresis is different from that of IC and eliminates many of the difficulties present in the early portion of an IC chromatogram. For example, sample matrix neutral organics, water, and cations do not interfere with anion analysis, and fluoride is well resolved from monovalent organic acids. Sample preparation typically is dilution with reagent water and removal of suspended solids by filtration. If necessary, hydrophobic sample components such as oil and grease can be removed with the use of HPLC solid-phase extraction cartridges without biasing anion concentrations.

4140 B. Capillary Ion Electrophoresis with Indirect UV Detection

1. General Discussion

a. Principle: A buffered aqueous electrolyte solution containing a UV-absorbing anion salt (sodium chromate) and an electro osmotic flow modifier (OFM) is used to fill a 75-µm-i.d. silica capillary. An electric field is generated by applying 15 kV of applied voltage using a negative power supply. This defines the detector end of the capillary as the anode. The sample is introduced at the cathodic end of the capillary and anions are separated on the basis of their differences in mobility in the electric field as they migrate through the capillary. Cations migrate in the opposite direction and are not detected. Water and neutral organics are not attracted toward the anode. They migrate after the anions and thus do not interfere with anion analysis. Anions are detected as they displace charge-for-charge the UV-absorbing electrolyte anion (chromate), causing a net decrease in UV absorbance in the analyte anion zone compared to the background electrolyte. Detector polarity is reversed to provide positive mv response to the data system (Figure 4140:1). As in chromatography, the analytes are identified by their migration time and quantitated by using time-corrected peak area relative to standards. After the analytes of interest are detected, the capillary is purged with fresh electrolyte, eliminating the remainder of the sample matrix before the next analysis.

b. Interferences: Any anion that has a migration time similar to the analytes of interest can be considered an interference. This method has been designed to minimize potential interference typically found in environmental waters, groundwater, drinking water, and wastewater.

Formate is a common potential interference with fluoride. It is a common impurity in reagent water, has a migration time similar to that of fluoride, and is an indicator of loss of water purification system performance and TOC greater than 0.1 mg/L. The addition of 5 mg/L to the mixed anion working standard aids in the identification of fluoride.

Generally, a high concentration of any one ion may interfere with the resolution of analyte anions in close proximity. Dilution in reagent water usually is helpful. Modifications in the electrolyte formulation can overcome resolution problems, but require individual validation for precision and bias. This method is capable of interference-free resolution of a 1:100 differential of Br^- to Cl^-, and NO_2^- and NO_3^- to SO_4^{2-}, and 1:1000 differential of Cl^- and SO_4^{2-}.

Dissolved ferric iron in the mg/L range gives a low bias for PO_4. However, transition metals do not precipitate with chromate because of the alkaline electrolyte pH.

c. Minimum detectable concentrations: The minimum detectable concentration for an anion is a function of sample size. Generally, for a 30-s sampling time, the minimum detectable concentrations are 0.1 mg/L (Figure 4140:2). According to the method for calculating MDL given in Section 1030 C, the calculated detection levels are below 0.1 mg/L. These detection levels can be compromised by analyte impurities in the electrolyte.

d. Limitations: Samples with high ionic strength may show a decrease in analyte migration time. This variable is addressed by using normalized migration time with respect to a reference peak, chloride, for identification, and using time-corrected area for quantitation. With electrophoresis, published data indicate that analyte peak area is a function of migration time. At high

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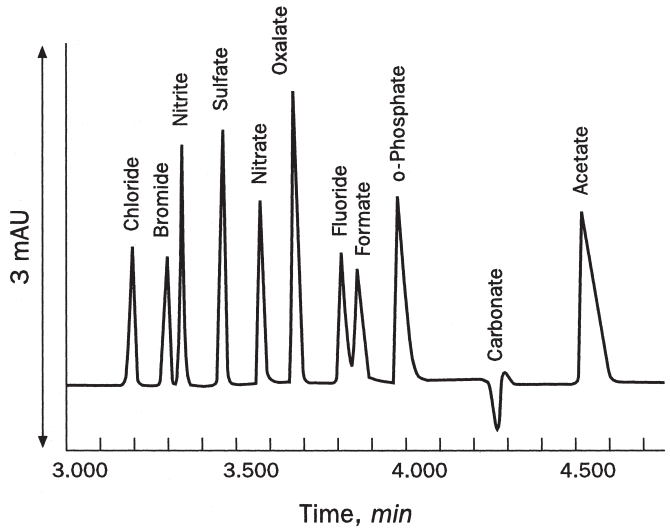

Figure 4140:1. Electropherogram of the inorganic anions and typically found organic acids using capillary ion electrophoresis and chromate electrolyte. Electrolyte: 4.7 mN Na_2CrO_4/4.0 mM TTAOH/10 mM CHES/0.1 mM calcium gluconate; capillary: 75-µm-i.d. × 375-µm-o.d. × 60-cm length, uncoated silica; voltage: 15 kV using a negative power supply; current: 14 ± 1 µA; sampling: hydrostatic at 10 cm for 30 s; detection: indirect UV with Hg lamp and 254-nm filter.

Anion	Conc (mg/L)	Migration Time (min)	Migration Time Ratio to Cl	Peak Area	Time-Corrected Peak Area
Chloride	2.0	3.200	1.000	1204	376.04
Bromide	4.0	3.296	1.030	1147	348.05
Nitrite	4.0	3.343	1.045	2012	601.72
Sulfate	4.0	3.465	1.083	1948	562.05
Nitrate	4.0	3.583	1.120	1805	503.69
Oxalate	5.0	3.684	1.151	3102	842.14
Fluoride	1.0	2.823	1.195	1708	446.65
Formate	5.0	3.873	1.210	1420	366.61
o-Phosphate	4.0	4.004	1.251	2924	730.25
Carbonate & bicarbonate		4.281	1.338		
Acetate	5.0	4.560	1.425	3958	868.01

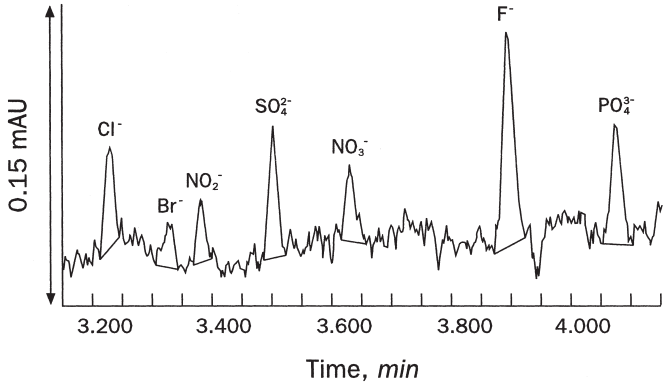

Figure 4140:2. Electropherogram of 0.1 mg/L inorganic anions at minimum detection level. Seven replicates of 0.1 mg/L inorganic anion standard used to calculate minimum detection levels, as mg/L, using analytical protocol described in Section 1030C.

Chloride	= 0.046	Nitrite	= 0.072
Nitrate	= 0.084	Phosphate	= 0.041
Bromide	= 0.090	Sulfate	= 0.032
Fluoride	= 0.020		

analyte anion concentrations, peak shape becomes asymmetrical. This phenomenon is typical and is different from that observed in ion chromatography.

2. Apparatus

a. Capillary ion electrophoresis (CIE) system: Various commercial instruments are available that integrate a negative high voltage power supply, electrolyte reservoirs, covered sample carousel, hydrostatic sampling mechanism, capillary purge mechanism, self-aligning capillary holder, and UV detector capable of 254-nm detection in a single temperature-controlled compartment at 25 °C. Optimal detection limits are attained with a fixed-wavelength UV detector with Hg lamp and 254-nm filter.

b. Capillary: 75-µm-i.d. × 375-µm-o.d. × 60-cm-long fused silica capillary with a portion of its outer coating removed to act as the UV detector window. Capillaries can be purchased premade or on a spool and prepared as needed.

c. Data system: HPLC-based integrator or computer. Optimum performance is attained with a computer data system and electrophoresis-specific data processing including data acquisition at 20 points/s, migration times determined at midpoint of peak width,

identification based on normalized migration times with respect to a reference peak, and time-corrected peak area.

3. Reagents

a. Reagent water: See Section 1080. Ensure that water is analyte-free. The concentration of dissolved organic material influences overall performance; preferably use reagent water with <50 µg/L TOC.

b. Chromate electrolyte solution: Prepare as directed from individual reagents, or purchase electrolyte preformulated.

1) *Sodium chromate concentrate,* 100 mM—In a 1-L volumetric flask dissolve 23.41 g sodium chromate tetrahydrate, $Na_2CrO_4 \cdot 4H_2O$, in 500 mL water and dilute to 1 L with water. Store in a capped glass or plastic container at ambient temperature. This reagent is stable for 1 year.

2) *Electroosmotic flow modifier concentrate,* 100 mM—In a 100-mL volumetric flask dissolve 3.365 g tetradecyl trimethyl ammonium bromide (TTAB), mol wt 336.4, in 50 mL water and dilute to 100 mL. Store in a capped glass or plastic container at ambient temperature. This reagent is stable for 1 year.

3) *Buffer concentrate,* 100 mM—In a 1-L volumetric flask dissolve 20.73 g 2-(N-cyclohexylamino)ethane sulfonate (CHES), mol wt 207.29, in 500 mL water and dilute to 1 L. Store in a capped glass or plastic container at ambient temperature. This reagent is stable for 1 year.

4) *Calcium gluconate concentrate,* 1 mM—In a 1-L volumetric flask dissolve 0.43 g calcium gluconate, mol wt 430.38, in 500 mL water and dilute to 1 L. Store in a capped glass or plastic container at ambient temperature. This reagent is stable for 1 year.

5) *Sodium hydroxide solution* (NaOH), 100 mM—In a 1-L plastic volumetric flask dissolve 4 g sodium hydroxide, NaOH, in 500 mL water and dilute to 1 L. Store in a capped plastic container at ambient temperature. This reagent is stable for 1 month.

6) *Chromate electrolyte solution*—Prerinse an anion exchange cartridge in the hydroxide form with 10 mL 100-mM NaOH followed by 10 mL water; discard the washings. Slowly pass 4 mL 100-mM TTAB concentrate through the cartridge into a 100-mL volumetric flask. Rinse the cartridge with 10 mL water and add to flask. Note: This step is needed to convert the TTAB from the bromide form into the hydroxide form TTAOH. The step can be eliminated if commercially available 100 mM TTAOH is used.

To the 100-mL volumetric flask containing the TTAOH add 4.7 mL sodium chromate concentrate, 10 mL CHES buffer concentrate, and 10 mL calcium gluconate concentrate. Mix and dilute to 100 mL with water. The pH should be 9 ± 0.1; final solution is 4.7 mM sodium chromate, 4 mM TTAOH, 10 mM CHES, and 0.1 mM calcium gluconate. Filter and degas through a 0.45-µm aqueous membrane, using a vacuum apparatus. Store any remaining electrolyte in a capped plastic container at ambient temperature for up to 1 month.

c. Standard anion solution, 1000 mg/L*:* Prepare a series of individual standard anion solutions by adding the indicated amount of salt, dried to constant weight at 105 °C, to 100 mL with water. Store in plastic bottles. These solutions are stable for 3 months. (Alternatively, purchase individual certified 1000-mg/L anion standards and store following the manufacturer's directions.)

Anion	Salt	Amount (g/100 mL)
Chloride	NaCl	0.1649
Bromide	NaBr	0.1288
Formate	$NaCO_2H$	0.1510
Fluoride	NaF	0.2210
Nitrite	$NaNO_2$	0.1499[a] (1000 mg NO_2^-/L = 304.3 mg NO_2^-–N/L)
Nitrate	$NaNO_3$	0.1371 (1000 mg NO_3^-/L = 225.8 mg NO_3^-–N/L)
Phosphate	Na_2HPO_4[b]	0.1500 (1000 mg PO_4^{3-}/L = 326.1 mg PO_4^{3-}–P/L)
Sulfate	Na_2SO_4[b]	0.1480 (1000 mg SO_4^{2-}/L = 676.3 mg SO_4^{2-}–S/L)

[a] Do not oven-dry, but dry to constant weight in a desiccator over phosphorous pentoxide.

[b] Potassium salts can be used, but with corresponding modification of salt amounts.

d. Mixed working anion standard solutions: Prepare at least 3 different working anion standard solutions that bracket the expected sample range, from 0.1 to 50 mg/L. Add 5 mg/L formate to all standards. Use 0.1 mL standard anion solution per 100 mL working anion solution (equal to 1 mg/L anion). (Above 50 mg/L each anion, chloride, bromide, nitrite, sulfate, and nitrate are no longer baseline-resolved. Analytes that are not baseline-resolved may give a low bias. If the analytes are baseline-resolved, quantitation is linear to 100 mg/L.) Store in plastic containers in the refrigerator. Prepare fresh standards weekly. Figure 4140:3 shows representative electropherograms of anion standards and Table 4140:1 gives the composition of the standards.

e. Calibration verification sample: Use a certified performance evaluation standard, or equivalent, within the range of the mixed working anion standard solutions analyzed as an unknown. Refer to Section 4020 B.2.

f. Analyte known-addition sample: To each sample matrix add a known amount of analyte and use to evaluate analyte recovery.

4. Procedure

a. Capillary conditioning: Set up the CIE system according to the manufacturer's instructions. Rinse the capillary with 100 mM NaOH for 5 min. Place freshly degassed chromate electrolyte solution into both reservoirs and purge the capillary with electrolyte for 3 min to remove all previous solutions and air bubbles. Apply a voltage of 15 kV and note the current. If the expected 14 ± 1 µA is observed, the CIE system is ready for use. Zero UV detector to 0.000 absorbance.

b. Analysis conditions: Program the CIE system to apply a constant current of 14 µA for the run time. Use 30 s hydrostatic sampling time for all standard and sample introduction. Analysis time is 5 min.

c. Analyte migration time calibration: Determine the migration time of each analyte daily using the midrange mixed working anion standard. Perform duplicate analysis to ensure migration time stability. Use the midpoint of the peak width, defined as midpoint between the start and stop integration marks, as the migration time for each analyte. This accounts for the observed nonsymmetrical peak shapes. (Use of peak apex may result in analyte misidentification.) The migration order is always Cl^-, Br^-, NO_2^-, SO_4^{2-}, NO_3^-, F^-, and PO_4^{3-}. Dissolved HCO_3^- is the last peak in the standard (see Figure 4140:1). Set analyte migration time window as 2% of the migration time determined above, except for Cl, which is set at 10%. Chloride is always the first

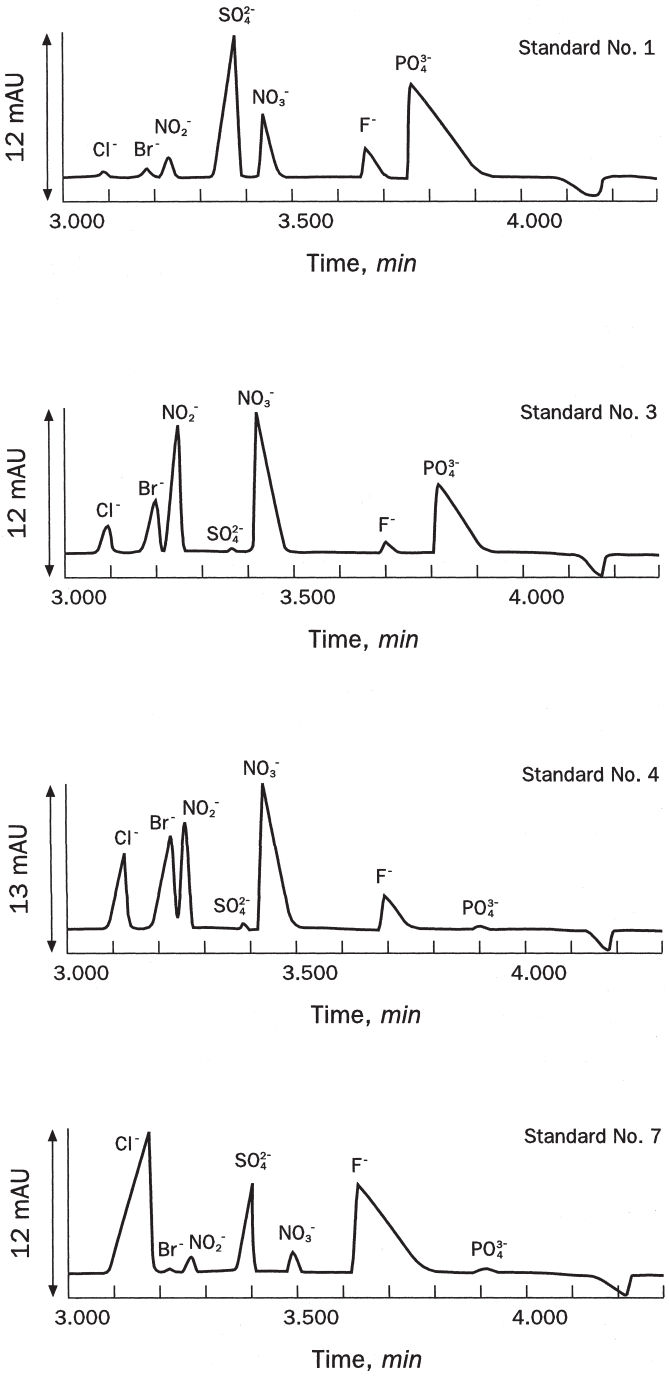

Figure 4140:3. Representative electropherograms of Youden anion standards. For composition of standards, see Table 4140:1.

Table 4140:1. Collaborative Design as Four Youden Pair Sets[a]

Anion	Anion Concentration in Individual Youden Pair Standards (mg/L)							
	1	2	3	4	5	6	7	8
Cl^-	0.7	2.0	3.0	15.0	40.0	20.0	50.0	0.5
Br^-	2.0	3.0	15.0	40.0	20.0	50.0	0.7	0.5
NO_2^-	3.0	40.0	20.0	15.0	50.0	0.5	2.0	0.7
SO_4^{2-}	40.0	50.0	0.5	0.7	2.0	3.0	15.0	20.0
NO_3^-	15.0	20.0	40.0	50.0	0.5	0.7	2.0	3.0
F^-	2.0	0.7	0.5	3.0	10.0	7.0	20.0	25.0
PO_4^{3-}	50.0	40.0	20.0	0.5	3.0	2.0	0.7	15.0

[a] The collaborative design is intended to demonstrate performance between 0.1 and 50 mg/L anion, except for fluoride between 0.1 and 25 mg/L. The concentrations among anions are varied so as not to have any one standard at all low or all high anion concentrations.

Table 4140:2. Anion Migration Time Reproducibility from Youden Pair Standards

Youden Standard	Anion Midpoint Migration Time, Average of Triplicate Samplings (min)						
	Cl^-	Br^-	NO_2^-	SO_4^{2-}	NO_3^-	F^-	PO_4^{3-}
1	3.132	3.226	3.275	3.405	3.502	3.761	3.906
2	3.147	3.239	3.298	3.431	3.517	3.779	3.931
3	3.138	3.231	3.283	3.411	3.497	3.771	3.925
4	3.158	3.244	3.307	3.434	3.510	3.781	3.963
5	3.184	3.271	3.331	3.435	3.551	3.787	3.981
6	3.171	3.260	3.312	3.418	3.537	3.776	3.964
7	3.191	3.272	3.315	3.437	3.544	3.773	3.978
8	3.152	3.248	3.294	3.418	3.526	3.739	3.954
SD[a]	0.021	0.015	0.018	0.012	0.20	0.015	0.027
%RSD[b]	0.67%	0.46%	0.55%	0.36%	0.56%	0.40%	0.68%

[a] Average SD = 0.018 min = 1.1 s.

[b] Average %RSD = 0.53%.

peak and is used as the reference peak for analyte qualitative identification. Identify anions on the basis of normalized migration times with respect to the reference peak, or migration time ratio. (See Figure 4140:1 and Table 4140:2.)

d. Analyte response calibration: Analyze all 3 mixed working anion standards in duplicate. Plot time-corrected peak area for each analyte versus concentration using a linear regression through zero. (In capillary electrophoresis peak area is a function of analyte migration time, which may change during analyses. Time-corrected peak area is a well-documented CIE normalization routine (i.e., peak area divided by migration time). (Note: Do not use analyte peak height.) Calibration is accepted as linear if regression coefficient of variation, R^2, is greater than 0.995. Linearity calibration curves for anions are shown in Figures 4140:4–6.

e. Sample analysis: After the initial calibration, run samples in the following order: calibration verification sample, reagent blank, 10 unknown samples, calibration verification sample, reagent blank, etc. Filter samples containing high concentrations of suspended solids. If peaks are not baseline-resolved, dilute the sample 1:5 with water and repeat analysis for unresolved analyte quantitation. Resolved analytes in the undiluted sample are considered correct quantitation. Electropherograms of typical samples are shown in Figures 4140:7–9.

5. Calculation

Relate the time-corrected peak area for each sample analyte with the calibration curve to determine the concentration of analyte.

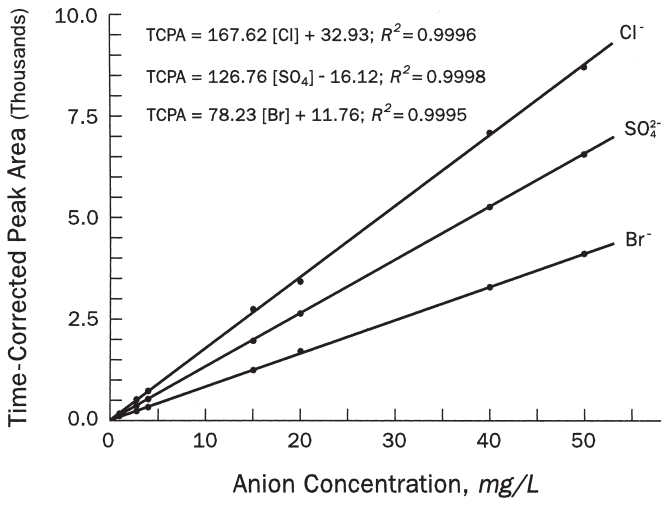

Figure 4140:4. Linearity calibration curve for chloride, bromide, and sulfate. Three data points were used per concentration; based on Youden pair design.

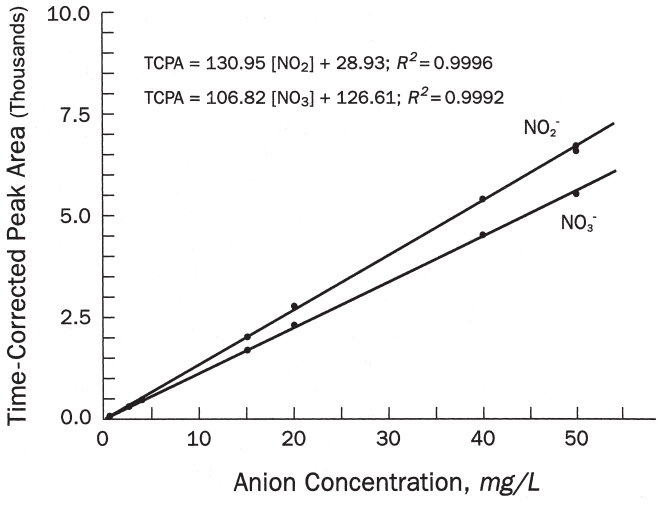

Figure 4140:6. Linearity calibration curve for nitrite and nitrate. Three data points were used per concentration; based on Youden pair design.

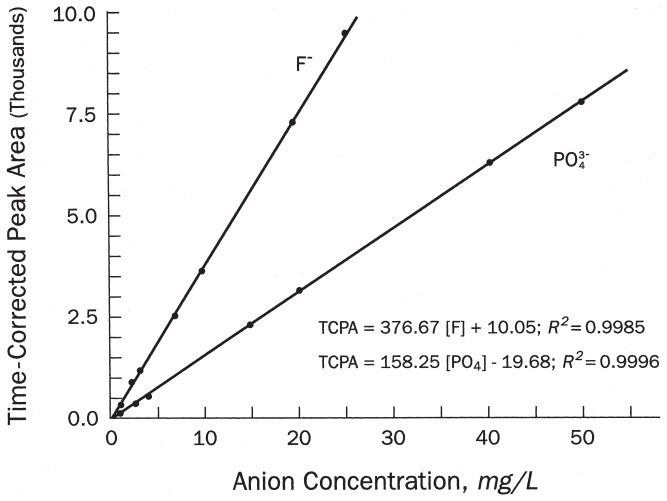

Figure 4140:5. Linearity calibration curve for fluoride and o-phosphate. Three data points were used per concentration; based on Youden pair design.

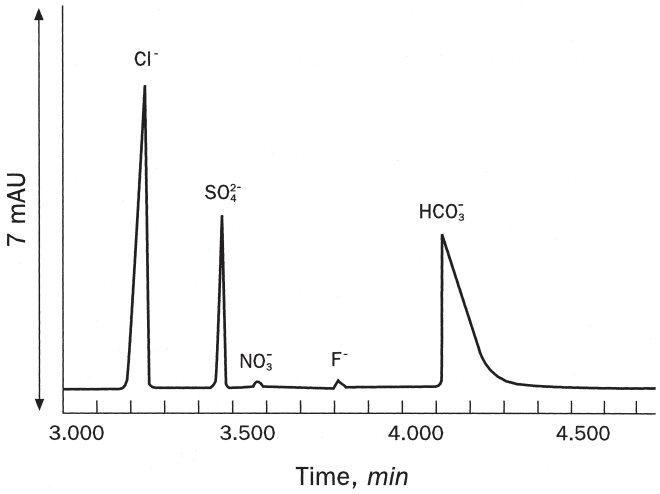

Figure 4140:7. Electropherogram of typical drinking water.

Chloride	= 24.72 mg/L	Fluoride < 0.10 mg/L
Sulfate	= 7.99 mg/L	Carbonate and bicarbonate = natural
Nitrate	= 0.36 mg/L	

If the sample was diluted, multiply the anion concentration by the dilution factor to obtain the original sample concentration, as follows:

$$C = A \times F$$

where:

C = analyte concentration in original sample (mg/L),
A = analyte concentration from calibration curve (mg/L), and
F = scale factor or dilution factor (for a 1:5 sample dilution, $F = 5$).

6. Quality Control

The QC practices considered to be an integral part of each method are summarized in Table 4020:1.

a. Analytical performance check: Unless an analyst has already demonstrated ability to generate data with acceptable precision and bias by this method, proceed as follows: Analyze 7 replicates of a certified performance evaluation standard containing the analytes of interest. Calculate the mean and standard deviation of these data. The mean must be within the performance evaluation standard's 95% confidence interval. Calculate percent relative